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ESTUDIO DE LA DIVERSIDAD DEL GÉNERO *PSEUDAMNICOLA* Y SU RADIACIÓN ENDÉMICA EN LA REGIÓN ÍBERO-BALEAR. TAXONOMÍA, FILOGENIA Y COROLOGÍA.

STUDY OF DIVERSITY IN THE GENUS *PSEUDAMNICOLA* AND ITS ENDEMIC RADIATION IN THE IBERO-BALEARIC REGION. TAXONOMY, PHYLOGENY AND CHOROLOGY

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Memoria presentada por Diana Delicado Iglesias para optar al grado de Doctor en Ciencias Biológicas por la Universidad Autónoma de Madrid.

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El tiempo no es oro, el tiempo es vida

José Luis Sampedro

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SUMMARY

The genus *Pseudamnicola* Paulucci, 1878 of the family Hydrobiidae Stimpson, 1865 comprises a group of very small freshwater snail species distributed throughout the Mediterranean basin. Two subgenera are generally recognized within the genus: *Pseudamnicola s. str.*, which is distributed throughout the Mediterranean basin, and *Corrosella* Boeters, 1970, which is only found in the Iberian Peninsula and southern France. *Corrosella* was initially described as genus but later placed within *Pseudamnicola*. Despite being one of the most diverse hydrobiid genera, there are few works that combine molecular analyses with detailed morphological and anatomical descriptions, mainly due to difficulties related to their small size and simplified anatomy. In view of this fact, this work constitutes the most comprehensive revision of the genus using an integrative approach that combines morphological, biogeographical and molecular analyses.

Previous works have demonstrated that the Ibero-Balearic region constitutes a hotspot of hydrobiid diversity. There are 13 valid species (11 of which are endemic) of the genus *Pseudamnicola* in this region. In 1988, Boeters revised several species of both subgenera in his monograph of the Ibero-Balearic hydrobiids: *P. (Corrosella) navasiana* (Fagot, 1907) and *P. (C.) hinzi* Boeters, 1986 from northern Iberia, *P. (C.) luisi* Boeters, 1984 and *P. (C.) falkneri* (Boeters, 1970) from southern Iberia, *P. (Pseudamnicola) subproducta* (Paladilhe, 1869) from several Iberian localities and the Balearic Majorca and Minorca Islands and *P. (P.) gasulli* Boeters, 1981 from southeastern Iberia and Ibiza Island. Boeters later also described a new species, *P. (C.) hydrobiopsis* Boeters, 1999, from the southern Iberian Peninsula. More recently, four new species of the nominotypical subgenus were described for the Balearic Islands, from localities formerly assigned to *P. (P.) subproducta*, thus questioning the presence of this species on this archipelago. Majorcan species were named *P. (P.) beckmanni* Glöer and Zettler, 2007, *P. (P.) granjaensis* Glöer and Zettler, 2007 and *P. (P.) artanensis* Altaba, 2007, and the Minorcan species was named *P. (P.) meloussensis* Altaba, 2007. Furthermore, *P. (C.) astieri* (Dupuy, 1851) from southern France and *P. (P.) conovula* (Frauenfeld, 1863), whose type locality is Pag Island (Croatia) though also was cited for Sardinia (Italy) and Tunisia, were also reported for the Iberian Peninsula.

Based on the original descriptions of these Ibero-Balearic species, sample collection was performed first by visiting the type localities, then the surrounding areas, and finally other regions for which the genus has been cited. In addition, previously collected samples available at the Museo Nacional de Ciencias Naturales were studied, as well as the type material preserved in other European museum collections. In total, approximately 150 Ibero-Balearic *Pseudamnicola* populations

were studied of which 128 belong to *P. (Corrosella)* and 30 to *P. (Pseudamnicola)*. For morphological and anatomical approaches, males and females from most localities were dissected and examined for commonly described hydrobiid characters related to the shell, penis, female genitalia, radula and nervous system, among other less common characters. Combined data from mitochondrial (16S rRNA and COI) and nuclear ribosomal (18S and 28S rRNA) fragments from 51 *P. (Corrosella)* and 20 Ibero-Balearic *P. (Pseudamnicola)* populations were genetically analyzed. Additional populations from other Mediterranean regions were also included in order to better understand the evolutionary history of the group. Phylogenetic and coalescence analyses were performed to examine patterns and causes of diversification. Furthermore, speciation factors such as water conductivity, altitude and geographic distances were studied by assessing the possible influence on speciation processes of environmental and geographical variables.

From a systematic point of view, both anatomical and molecular analyses revealed: i) the existence of seven new species of *P. (Corrosella)* from the Iberian Peninsula; ii) that the Iberian records formerly ascribed to *P. (C.) astieri* actually correspond to a new cryptic species, *P. (C.) hauffei* Delicado and Ramos, 2012; iii) the validation of the Balearic species as different taxa from *P. (P.) subproducta*; iv) that *P. (P.) conovula* does not seem to occur in the Ibero-Balearic region; v) the genus *Pseudamnicola* should be considered composed of three genera: *Pseudamnicola s. str.*, *Corrosella* and a new genus with *P. (P.) gasulli* as the type species. Overall, the most valid traits for differentiating between the studied species formerly included within the genus *Pseudamnicola* are: shell length/shell width ratio, the shape and length of the seminal receptacle, the length of the bursa copulatrix and bursal duct, the pigment extension on the renal oviduct, the shape and dimensions of the penis and the dimensions of the prostate gland.

Phylogenetically, these three genera were monophyletic in all analyses; however, the evolutionary relationships among them still remain uncertain. The reestablished genus *Corrosella* is composed of 12 Iberian species and *C. astieri* from southern France, clustered in three well-supported lineages, which grouped the northern species in two clades and the southern ones in a third clade (which was further divided into two clades in coalescence analyses). Speciation processes that influenced these diversification patterns may be related to habitat fragmentation and isolation, which may have occurred in three independent events during the Miocene (approximately 10 Ma, 5 Ma and 2 Ma). Within the Ibero-Balearic *Pseudamnicola s. str.* spp., the Minorcan species *P. meloussensis* is clustered with the Iberian species *P. subproducta*, whereas the three Majorcan species are comprised within a unique clade. Dating studies showed that the separation of the Balearic Islands from the continent occurred prior to the divergence between Iberian and Balearic species, and therefore, the presence of this group on the islands is likely related to two later

transmarine colonizations followed by vicariance. This speciation mechanism may be common within *Pseudamnicola s. str.* spp., as the pattern of genetic variation reflects dispersal factors associated with this group. Furthermore, coalescence analyses showed evidence for a possible radiation of this genus mainly during the Messinian Salinity Crisis (5.9-5.3 Ma). During this, the conditions were ideal for dispersal processes in terrestrial fauna, with the subsequent interruption of the gene flow due to the aperture of Gibraltar Strait and the filling of the Mediterranean Basin. This combination of factors may have caused the radiation phenomenon in the group.

Overall, statistical tests revealed that diversification patterns of *Pseudamnicola s. l.* spp. were strongly related to habitat fragmentation or dispersion events rather than to environmental conditions. However, habitat-related factors seem to have played a larger role during the deep divergent split leading to the three genera. Furthermore, these three groups display different evolutionary patterns caused by differences in tempo and mode of diversification, which may be related to habitat: *Corrosella* spp. occur in isolated springs and stream headwaters of mountainous regions, whereas species belonging to *Pseudamnicola* and the new genus inhabit brackish streams, lagoons and low river stages where waters remain connected, thus allowing gene flow between populations.

In conclusion, by taking an integrative approach, this thesis provides a better understanding of the systematics and evolution of the family Hydrobiidae, which is essential for managing and preserving the species current habitats and populations.

INTRODUCCIÓN

SISTEMÁTICA DE LA FAMILIA HYDROBIIDAE STIMPSON, 1865

* Phylum Mollusca

Clase Gastropoda

Subclase Orthogastropoda

Clado Caenogastropoda

Clado Hypsogastropoda

Superfamilia Rissooidea Gray, 1847

Familia Hydrobiidae Stimpson, 1865

* Clasificación según la propuesta de Bouchet y Rocroi, 2005

La familia Hydrobiidae Stimpson, 1865 reúne un conjunto de moluscos gasterópodos prosobranquios de pequeño tamaño (de 1 a 8 mm) que habitan principalmente en ecosistemas dulceacuícolas, aunque existen algunos géneros de aguas salobres, como *Hydrobia* Hartmann, 1821 o *Peringia* Paladilhe, 1874. Teniendo en cuenta la dificultad existente para definir los límites entre los diferentes estatus taxonómicos en la sistemática de gasterópodos, Kabat y Hershler (1993) estimaron en 400 el número de géneros y en 1.000 el número de especies nominales que componen esta familia. Años después, este número fue recalculado hasta un total de 1.250 especies (Strong *et al.*, 2008), considerando ambas estimaciones el carácter cosmopolita de la familia Hydrobiidae. Sin embargo, recientemente Wilke *et al.* (2013), a través del estudio de filogenias moleculares, han establecido los géneros dentro de la superfamilia Rissooidea Gray 1847 que conformarían la familia Hydrobiidae, considerando a los hidróbidos *sensu stricto* (*s. str.*) como un grupo monofilético. De acuerdo con este último estudio, los hidróbidos *s. str.* tendrían una distribución más restringida, estando presentes en la región occidental del Paleártico, el este del Neártico, el norte del Neotrópico y Sudáfrica.

Además de ser una de las familias más abundantes de moluscos de agua dulce, se considera que Hydrobiidae es una de las más ancestrales, ya que está datada en el registro fósil desde principios del Carbonífero, aproximadamente hace 280 Ma (Knight *et al.*, 1960; Solem y Yochelson, 1979). No obstante, Ponder (1988) cuestionó la identificación de esos ejemplares como hidróbidos, atribuyéndolos a la familia Ampullariidae.

En relación a las categorías taxonómicas superiores, la clasificación de la familia Hydrobiidae es todavía confusa, al igual que los límites y las relaciones interespecíficas de los taxones que la componen. Tanto es así que esta familia ha sido catalogada dentro de tres superfamilias distintas: Rissooidea (Thiele, 1929; Wenz, 1939; Taylor y Sohl, 1962; Taylor, 1966; Bernasconi, 1992), Hydrobioidea (Radoman, 1973, 1983) y Truncatelloidea (Golikov y Starobogatov, 1975; Ponder y Warén, 1988). El pequeño tamaño y la escasa escultura de las conchas, en su mayoría lisas, hacen muy difícil obtener caracteres taxonómicos útiles. Además, la convergencia evolutiva de la forma de la concha de los gasterópodos hidrobioides (término informal acuñado por Davis en 1979 para referirse a los gasterópodos rissooideos que comparten las características generales de hidróbidos) parece un hecho probado. Por estas razones, aún no está claramente establecido el carácter monofilético de la familia, y la distinción entre los hidróbidos y otras familias de rissooideos no está bien definida. Las clasificaciones propuestas hasta el momento están basadas en escasos caracteres morfológicos o en pequeños grupos de datos moleculares, por lo que la sistemática de este grupo es aún objeto de estudio. A continuación, se resume la clasificación de la familia Hydrobiidae dentro de Rissooidea a lo largo de la historia.

Troschel (1857) definió Hydrobiidae como un grupo de moluscos con un "rango de incertidumbre" pertenecientes al orden Ctenobranchiata. Sin embargo, la autoría de la familia ha sido recientemente atribuida a Stimpson (1865) (Bouchet y Rocroi, 2005), debido a que Troschel no encontró caracteres morfológicos suficientes para establecer el rango de familia para algunos géneros, los cuales, consecuentemente, fueron denominados no con la terminología correspondiente a la categoría de familia, sino como Bithyniae, Hydrobiae, Litogliphy, Pachichily y Thiarae. Así, según Bouchet y Rocroi (2005), el nombre de Hydrobiae fue considerado como una referencia temporal sin categoría taxonómica de familia, y el primer autor que trató a Hydrobiidae como tal fue Stimpson (1865). Todavía recientemente existían problemas nomenclaturales con el nombre de la familia, al ser éste considerado un homónimo anterior de la familia Hydrobiidae Mulsant, 1844 (Coleoptera). De las múltiples alternativas sugeridas para establecer el nombre de la familia, cabe destacar la de Giusti *et al.* (1998), que solicitaron al Comité Permanente de la Comisión Internacional de Nomenclatura Zoológica (CINZ) mantener Hydrobiidae para Mollusca (género tipo: *Hydrobia*), e Hybrobiusina para Insecta Coleoptera (género tipo: *Hydrobius* Mulsant, 1844). Esta solicitud fue aprobada por la CINZ (2003a, Opinión 2034) e Hydrobiidae fue incluido en la Lista Oficial de nombres, si bien atribuido a Troschel, 1857 como autor, al haber sido admitido con anterioridad a la publicación de Bouchet y Rocroi (2005, p. 6).

Tabla 1. Algunas clasificaciones previas de la familia Hydrobiidae. Los taxones asignados a la familia Hydrobiidae se muestran en negrita. Los interrogantes indican posibles clasificaciones hipotéticas para esos taxones. Tomado de Wilke *et al*. (2001).

Taylor (1966)	Davis (1979)	Radoman (1983)	Starobogatov and Sitnikova (1983)	Ponder and Warén (1988)	Bernasconi (1992)	Kabat and Hershler (1993)
Hydrobiidae	Bithyniidae	Bythinellidae	Amnicolidae	Bithyniidae	Bythiniidae	Bithyniidae
Amnicolinae	**Hydrobiidae**	**Hydrobiidae**	Bithyniidae	**Hydrobiidae**	**Hydrobiidae**	**Hydrobiidae**
Cochliopinae	**Hydrobiinae**	**Hydrobiinae**	Horatiidae	**Hydrobiinae**	**Hydrobiinae**	**Amnicolinae (?)**
Cochliopini	Pomatiopsidae	**Pseudamnicolinae**	Orientalininae	**Littoridininae**	**Horatiini**	**Cochliopinae**
Horatiini	Rissoidae	Lithoglyphidae	Horatiinae	**Lithoglyphinae**	**(= Islamiinae)**	**(= Littoridininae)**
Hydrobiinae	Truncatellidae	Orientalinidae	**Hydrobiidae**	**Nymphophilinae**	**Hydrobiini**	**Hydrobiinae**
Lithoglyphinae		Orientalininae	Islamiidae	**Moitessieriinae**	**(= Pseudamnicolinae)**	**Islamiinae (?)**
Littoridininae		Islamininae	Littoridinidae	**Amnicolinae**	**Nymphophilini**	**Lithoglyphinae**
Nymphophilinae			Moitessieriidae	Pomatiopsidae	**Lithoglyphini**	**Moitessieriinae (?)**
Pomatiopsinae			Pomatiopsidae	Rissoidae	**Moitessieriini**	**Nymphophilinae**
Bithyniidae			Sadlerianidae	Truncatellidae	**Amnicolinae**	**Sadlerianinae (?)**
			Pseudamnicolinae		**Littoridininae (?)**	Pomatiopsidae
			Sadlerianinae		Pomatiopsidae	Rissoidae
			Triculidae		Truncatellidae	Truncatellidae
			Pomatiopsidae			

Aparte de Stimpson, otros autores del siglo XIX (Tryon, 1866; Fischer, 1885) situaron a los hidróbidos como una familia cercana a Rissooidea Gray, 1847 (e incluso formando parte de ella). Este hecho resulta comprensible, ya que en aquella época las descripciones estaban basadas en algunos caracteres de la concha, del opérculo, del diente central de la rádula, del pie y del pene (Kabat y Hershler, 1993). Ya en el siglo XX, Thiele trató a la familia en varias ocasiones (Thiele 1925, 1928, 1929), pero sus diagnosis estaban basadas principalmente en la concha y en la rádula, con un mínimo uso de los caracteres anatómicos. Incluyó siete subfamilias dentro de la familia Hydrobiidae, consideradas hoy en día como familias independientes. Este trabajo, junto con el de Wenz (1939), que utilizó la clasificación de Thiele, constituye uno de los trabajos más exhaustivos publicados para la familia a nivel de género.

Años después, Morrison (1949) dividió a la familia Hydrobiidae (a la que denominó Amnicolidae Tryon, 1863 en su clasificación) en varias subfamilias en función del número de conductos penianos (y, en menor medida, según la forma del opérculo). Esta organización no ayudó demasiado a diferenciar entre los grupos de hidróbidos, puesto que la mayoría de los rissooideos tienen un único conducto peniano. En un trabajo posterior, Morrison (1971) incluyó 16 subfamilias nominales en este grupo, criterio que consiguió más aceptación en las siguientes décadas. Uno de los trabajos que comenzó a emplear un mayor número de caracteres, como los tentáculos cefálicos, la pigmentación y la reproducción, fue el de Taylor (1966) (ver Tabla 1). Sin embargo, este trabajo fue criticado, ya que trataba únicamente sobre especies del "Nuevo Mundo", y algunas de las subfamilias, según Thompson (1968), estaban débilmente diferenciadas y la importancia de algunos caracteres clave estaba sobreestimada.

Radoman (1969) comenzó a incluir otros caracteres anatómicos y a cuestionar la validez de las descripciones basadas únicamente en características conquiliológicas. En una clasificación de la fauna del este de Europa, Radoman (1973, 1983, 1985) empleó caracteres como el diente central de la rádula, la presencia o ausencia del ciego gástrico del estómago, la longitud de los conectivos nerviosos, el aparato genital femenino y el pene. En su trabajo de 1983 clasificó a los Hydrobioideos europeos en seis familias, 83 géneros y 190 especies (Tabla 1). A pesar de tratarse de revisiones mucho más completas, la obra de Radoman fue cuestionada posteriormente por otros autores (Davis, 1978, 1982; Giusti y Pezzoli, 1984) alegando que esta clasificación estaba demasiado dividida y los grupos muchas veces no estaban bien diferenciados. Otra monografía de revisión de la familia Hydrobiidae, la cual destacó también la importancia del aparato genital femenino en las diagnosis, fue la de Starobogatov (1970), quien utilizó en sus descripciones caracteres como el oviducto renal y glandular y las bolsas espermáticas.

En un estudio similar, Giusti y Pezzoli (1982) clasificaron a los hidróbidos en dos superfamilias diferentes (Hydrobioidea y Pyrguloidea) basándose en la presencia o ausencia de los márgenes laterales y de las cúspides basales del diente radular central. En 1988, Ponder y Warén (1988) retornaron a los hidróbidos a la superfamilia Rissooidea, dividiéndola en tres familias (ver Tabla 1). Bernasconi (1992) realizó un estudio de clasificación de los Rissooidea no marinos, dividiéndolos en dos grupos: los hidrobioides, compuestos por cuatro familias: Bithyniidae Gray, 1857, Hydrobiidae, Pomatiopsidae Stimpson, 1865 y Truncatellidae Gray, 1840 (Tabla 1), y los asimineidos, compuestos por otras tres familias de gasterópodos.

El hecho de que todas estas clasificaciones estén basadas en aproximaciones puramente anatómicas en las que no se incluye ningún estudio filogenético refleja que, hasta finales del siglo XX, no se tuvo en cuenta el tratamiento evolutivo de los caracteres anatómicos. Cuando se ha tenido en cuenta este marco evolutivo, el origen monofilético de la familia ha sido cuestionado en varias ocasiones (Kabat y Hershler, 1993; Wilke *et al.*, 2001; Arconada y Ramos, 2003). Ponder (1988) fue uno de los primeros en proporcionar una filogenia para las familias de rissooideos basada en análisis cladísticos. En esta filogenia, los hidróbidos aparecen como un clado de posición intermedia, ya que es un grupo más reciente en comparación con otras familias marinas de rissooideos, pero es uno de los grupos de agua dulce más ancestrales. Además, Ponder mostró la dificultad de resolver las relaciones filogenéticas entre los grandes clados de Rissooidea, debido a que los cambios de carácter que definen las ramas y los nodos basales muestran reversiones y paralelismos (homoplasias) y a que el número de caracteres únicos que podrían agrupar satisfactoriamente a los taxones (sinapomorfías) es relativamente pequeño.

En un intento de unificar la terminología para la descripción de los caracteres morfológicos y aplicarla en futuras filogenias, Hershler y Ponder (1998) publicaron una monografía donde revisaron exhaustivamente la mayoría de los caracteres y estados de carácter susceptibles de ser empleados en las descripciones de nuevos taxones de hidróbidos. Así, en este trabajo se incluyeron descripciones de caracteres de la concha, del opérculo, de la cabeza, del pie, de la rádula, del sistema digestivo, de los órganos paleales y de los órganos genitales masculinos y femeninos. A día de hoy, esta terminología es la más empleada en estudios morfológicos sobre la familia Hydrobiidae *sensu lato* (*s. l.*) (entre ellos Ramos *et al*., 2000; Arconada y Ramos, 2001, 2006; Hershler, 2001; Szarowska *et al.*, 2007) y es, de hecho, la utilizada en las descripciones morfológicas de esta tesis.

A pesar de que la revisión de Hershler y Ponder (1998) estableció una buena base morfológica para las descripciones de hidróbidos, los estudios cladísticos basados únicamente en datos morfológicos han dado como resultado filogenias

incongruentes, con un alto contenido en caracteres homoplásicos (Falniowsky y Szarowska, 2000; Bodon *et al.*, 2001; Szarowska, 2006; Haase, 2008). En un primer intento de combinar los estudios genéticos con los datos anatómicos, Wilke *et al.* (2001) analizaron el origen monofilético de la familia Hydrobiidae a partir de la clasificación dada por Kabat y Hershler (1993). La nueva filogenia se obtuvo a partir de la combinación de secuencias procedentes del gen mitocondrial citocromo c oxidasa subunidad I (COI) y del gen nuclear ARNr 18S obtenidas de varias familias de Rissooidea. Dicho estudio fue apoyado anatómicamente, comparando la organización general del sistema genital femenino de cada una de las familias. Como resultado, se pudo confirmar la monofilia de la familia Hydrobiidae *s. str.*, esto es, asumiendo como grupos diferentes, previamente incluidos en Hydrobiidae *s. l.*, a las familias Moitessieriidae Bourguignat, 1863, Bithyniidae, Lithoglyphidae Tryon, 1866, Cochliopidae Tryon, 1866 y Amnicolidae (Tabla 2).

Más recientemente, Bouchet y Rocroi (2005) publicaron la clasificación de moluscos gasterópodos que cuenta actualmente con mayor consenso y que es la utilizada en este trabajo. Debido a que la filogenia de los gasterópodos está actualmente en una fase activa de reevaluación, la clasificación de las altas jerarquías taxonómicas por encima del nivel de superfamilia no es estable y, por ello, emplearon el término "clado" para catalogar a aquellas categorías que no están claramente resueltas.

Los últimos estudios publicados hasta el momento sobre la superfamilia Rissooidea y la situación de Hydrobiidae dentro de la misma han sido desarrollados a una escala más regional. Uno de estos trabajos es el de Boeters (1998), en el cual se detallan y describen, principalmente con caracteres conquiliológicos y de los sistemas genitales masculino y femenino, las familias de Rissooidea de Europa Central (ver Tabla 2). Años más tarde, Szarowska (2006), a través de una filogenia molecular (combinando los genes COI y ARNr 18S) y el estudio evolutivo de los caracteres morfológicos, analiza también las familias de Rissooidea en los Balcanes, concluyendo que la familia Hydrobiidae está compuesta únicamente por dos subfamilias (Hydrobiinae y Sadlerianiinae) y que su grupo más cercano filogenéticamente lo constituye la familia Assimineidae Adams y Adams, 1856, y no Moitessieriidae o Bithyniidae, como apuntaban Wilke *et al.* (2001). Ponder *et al.* (2008) publicaron la que posiblemente sea una de las filogenias más completas hasta el momento de la superfamilia Rissoidae en Australia. A través del estudio de los genes COI, ARNr 16S y 18S en más de 40 géneros pudieron definir molecularmente algunas de las familias australianas de estos gasterópodos. Además, pudieron demostrar la amplia distancia genética existente entre los géneros de hidróbidos australianos y los descritos para la región mediterránea y Norteamérica, postulando así la hipótesis filogenética que fue apoyada posteriormente por los resultados del trabajo de Wilke *et al.* (2013) (Tabla 2).

Tabla 2. Clasificaciones más recientes de la familia Hydrobiidae dentro de la superfamilia Rissooidea. Los taxones asignados a la familia Hydrobiidae se muestran en negrita. Los interrogantes indican posibles clasificaciones hipotéticas para esas categorías.

Boeters, 1998	Wilke *et al.*, 2001	Bouchet y Rocroi, 2005	Wilke *et al.*, 2013
Bithyniidae	Rissoidae	Rissoidae	**Hydrobiidae**
Moitessieriidae	**Hydrobiidae**	Amnicolidae	**Nymphophilinae**
Hydrobiidae	**?**	Anabathridae	**?**
Emmericiinae	**Horatiinae?**	Assimineidae	**Pseudamnicolinae**
Lithoglyphinae	**?**	Barleeiidae	**Hydrobiinae**
Potamopyrginae	**Islamiinae?**	Bithyniidae	**Islamiinae**
Cocliophinae	**Pseudamnicolinae**	Caecidae	**Belgrandielinae**
Hydrobiinae	**Hydrobiinae**	Calopiidae	**Belgrandinea**
Horatiinae	**Nymphophilinae**	Cochliopidae	**Horatiinae**
Amnicoliinae	Moitessieriidae	Elachisinidae	Rissoidae
	Bithyniidae	Emblandidae	Moitessieriidae
	Lithoglyphidae	Epigridae	Bithyniidae
	Cochliopidae	Falsicingulidae	Lithoglyphidae
	Amnicolidae	Helicostoidae	Cochliopidae
	Pomatiopsidae	**Hydrobiidae**	Amnicolidae
	Truncatellidae	Hydrococcidae	Pomatiopsidae
		Iravadiidae	Truncatellidae
		Lithoglyphidae	Emmericidae
		Moitessieriidae	“Fontigentinae”
		Pomatiopsidae	Clenchielidae
		Stenothyridae	Eulimidae
		Tornidae	Eatoniellidae
		Truncatellidae	Littorinidae
			…

EL CONCEPTO DE ESPECIE EN HIDRÓBIDOS

Aunque los hidróbidos son uno de los grupos más abundantes en los ambientes de agua dulce, la mayoría de sus especies están restringidas a un escaso número de localidades o ecosistemas frágiles de aguas permanentes (p. ej., manantiales, fuentes, arroyos, lagunas, etc.), considerablemente amenazados por factores humanos o por la escasez de agua, principalmente. Por consiguiente, con el fin de preservar la fauna de hidróbidos y antes de tomar medidas de conservación, es necesario tener un conocimiento de la diversidad del grupo, ya que, en la práctica, el número de especies actualmente conocidas es posible que diste bastante del número real de especies existentes (Strong *et al.*, 2008). Uno de los principales motivos de este hecho es la dificultad de establecer los límites morfológicos y moleculares de cada entidad taxonómica y aplicar así un concepto de especie en hidróbidos o un criterio para determinarlas.

Desde el punto de vista morfológico, estas especies pequeñas e incluso diminutas parecen presentar caracteres cuya evolución incluye procesos de paralelismo y convergencia (homoplasias) y no del hecho de compartir un mismo origen evolutivo (homologías). Así, la mayor parte de los caracteres conquiliológicos, la presencia o ausencia de ojos y de pigmentación corporal (asociadas respectivamente a ambientes superficiales o intersticiales o cavernícolas), el número de laminillas branquiales del ctenidio, etc., son algunos ejemplos de esos caracteres homoplásicos que dificultan la identificación de las especies o, al menos, el establecimiento de sus relaciones filogenéticas. A esta circunstancia se añaden otros elementos, como la simplificación estructural debido a la miniaturización, el relativamente alto grado de variabilidad intraespecífica de los caracteres anatómicos, la ausencia de datos paleontológicos y, por lo tanto, el desconocimiento de la historia evolutiva de la familia, que dificultan la obtención de caracteres diagnósticos efectivos para la descripción de las especies. Así, ya que la aproximación morfológica por sí sola no puede resolver la sistemática del grupo, se requiere el apoyo de otras disciplinas como la genética, la ecología o la biogeografía.

Consecuentemente, entre todos los conceptos de especie propuestos hasta el momento (biológico, geográfico, filogenético, evolutivo, ecológico, etc.) en este trabajo se ha empleado el "concepto filogenético de especie" (Rosen, 1978; Cracraft, 1983; Nixon y Wheeler, 1990; Davis y Nixon, 1992), que aplica un enfoque filogenético para establecer los límites entre especies. De acuerdo con ese concepto, se entiende por especie un conjunto de poblaciones con un origen evolutivo común y que puede ser diagnosticada por poseer una combinación única de estados de carácter morfológicos y genéticos. Este concepto fue acuñado originalmente en el trabajo de Hennig (1966), base de la escuela filogenética (cladística) actual.

Ponder (1988), como se explicaba en la sección anterior, fue uno de los primeros autores en proporcionar una filogenia de las familias de Rissooidea basada en análisis cladísticos. Sin embargo, la dificultad encontrada por este autor para resolver las relaciones filogenéticas entre los grupos de jerarquía superior dentro de los Rissooidea, se refleja en el hecho de que muchas de las transformaciones de los estados de carácter que definen las ramas o nodos del cladograma son reversiones o paralelismos y no caracteres derivados (sinapomorfías), los cuales son escasos. Incluso Kabat y Hershler (1993) reconocieron que las dos autopomorfías que definen a la familia Hydrobiidae (pérdida de los tentáculos paleales y de las glándulas penianas) han evolucionado dentro de la familia, ya que *Hydrobia*, género considerado como el más primitivo de la familia, conserva los tentáculos paleales y otros taxones presentan glándulas penianas.

Como se ha indicado, uno de los conflictos para determinar el número real de taxones y, por lo tanto, demostrar la monofilia de la familia, se debe a la dificultad para reconocer los límites entre las especies de hidróbidos, ya que la mayoría de ellas tienen conchas sin escultura y presentan una simplificación en sus estructuras anatómicas (Arconada y Ramos, 2003). Estas características conllevan altos niveles de homoplasia y de existencia de especies crípticas (p. ej., Liu *et al*., 2003; Haase, 2008). Es por ello que los últimos trabajos publicados para hidróbidos ya utilizan un doble enfoque, combinando descripciones morfológicas más completas con datos moleculares (p. ej., Szarowska *et al.*, 2006; Szarowska, 2006; Haase, 2008; Hershler y Liu, 2009). La mayoría de los estudios moleculares han basado sus inferencias filogenéticas en dos tipos de secuencias: genes mitocondriales, principalmente el gen COI (Davis *et al.*, 1998; Wilke y Davis, 2000; Wilke *et al.*, 2001; Wilke y Falniowski, 2001; Hershler *et al.*, 2003; Szarowska, 2006; Hershler y Liu, 2009; Delicado *et al.*, 2012; Delicado y Ramos, 2012; Wilke *et al.*, 2013), y el gen nuclear ARNr 18S (Wilke *et al.*, 2000b; Wilke *et al.*, 2001; Szarowska *et al*., 2005; Wilke *et al.*, 2013).

Sin embargo, las filogenias publicadas para hidróbidos que combinan datos morfológicos y moleculares tampoco resuelven completamente las relaciones filogenéticas entre los taxones. Por ello, no sólo es necesaria la búsqueda de caracteres morfológicos útiles que caractericen correctamente a las especies, sino también la exploración de los marcadores moleculares que mejor definan las relaciones filogenéticas entre ellas. Esta cuestión es uno de los objetivos principales de la presente tesis.

LA REGIÓN ÍBERO-BALEAR COMO PUNTO CALIENTE ("HOTSPOT") DE DIVERSIDAD PARA LAS ESPECIES DE HIDRÓBIDOS

A escala global se estima que existen alrededor de unas 8.000 especies de gasterópodos de agua dulce (Strong *et al.*, 2008). Se considera que, mientras que el número de familias que se conocen llega casi al 90% de las realmente existentes, dentro de los hidrobioides sólo se ha descrito el 25% de su diversidad específica real (Strong *et al.*, 2008). Con una distribución cosmopolita, los hidrobioides (Hydrobiidae *s. l.*) han colonizado todos los continentes, aunque en determinadas regiones la diversidad y el número de taxones endémicos es más alto que en otras. Una de estas áreas de alta diversidad o punto caliente ("hotspot") de gasterópodos de agua dulce es el conjunto de las regiones montañosas de la península Ibérica y del sur de Francia (Figura 1). Este punto caliente de diversidad está dominado principalmente por hidrobioides, estimándose en 150 su número de especies, 140 de las cuales son endémicas (Strong *et al.*, 2008).

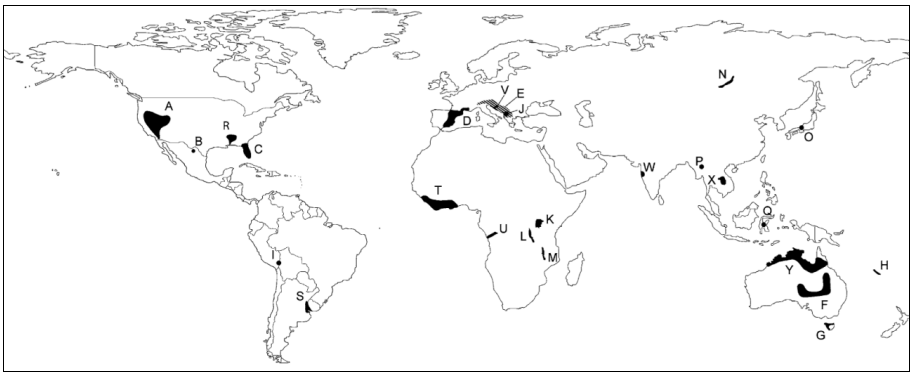

Figura 1. Puntos calientes de diversidad de gasterópodos de agua dulce en el mundo, según Strong *et al.* (2008). **A-H.** Fuentes y aguas subterráneas. **I-Q.** Lagos. **R-X.** Ríos. **Y.** Humedales monzónicos.

En concreto, la península Ibérica se considera uno de los "centros evolutivos" para las especies de hidróbidos, junto con las penínsulas Itálica y Balcánica (Davis, 1982). Esto puede deberse a la combinación de varios factores, como el elevado relieve de estas regiones, ya que las montañas podrían actuar como una barrera zoogeográfica, una extensa red fluvial consecuencia de ese acusado relieve, la situación de puente de la península Ibérica entre las influencias atlántica y mediterránea y entre los continentes europeo y africano, la variedad microclimática debida a los factores anteriores, el efecto refugio producido durante las glaciaciones

en el sur de Europa, y la escasa capacidad de dispersión de estos gasterópodos (Arconada y Ramos, 2003). Además, a estos factores se les puede añadir el hecho de que los hidróbidos viven en hábitats muy restringidos, generalmente en pequeñas masas de agua (fuentes, acequias, arroyos, lagos, etc.) bien oxigenadas y de corriente permanente; por ello, se les considera indicadores de calidad de las aguas. Todos estos factores podrían ser la causa del alto número de especies endémicas de hidróbidos existentes en la región íbero-balear, y serán tratados y evaluados como posibles causas de los patrones evolutivos encontrados para el género estudiado en esta tesis.

A pesar del gran número de especies, existe una bibliografía relativamente escasa acerca de la familia Hydrobiidae en la región íbero-balear. A finales del siglo XIX y durante el siglo XX se publicaron algunos trabajos a escala local, la mayoría de ellos revisiones conquiliológicas: Rosenhauer (1856), Fagot (1887, 1905, 1907), Bofill (1909), Bofill y Haas (1920), Haas (1924, 1925, 1927), Gasull (1965, 1971, 1981), Altimira (1960), Schütt (1961), Boeters (1969) y Bech (1990). Boeters (1988) publicó una revisión de las familias Hydrobiidae y Moitessieriidae en la región íbero-balear, en la que se estudiaron 35 especies de hidróbidos. Las publicaciones más recientes (Ramos *et al.*, 1992; Arconada *et al.*, 1996) demuestran que las cifras aportadas por Boeters (1988) representan aproximadamente un 50% de las especies de hidróbidos conocidas hasta ahora en los grupos revisados más exhaustivamente. Partiendo de la hipótesis de que no todas las especies de hidróbidos de esta región están incluidas en el trabajo de Boeters, uno de los objetivos de la presente tesis doctoral será revisar las especies del género *Pseudamnicola* Paulucci, 1878 publicadas por dicho autor en ése y en otros de sus trabajos. La necesidad de revisar el estudio de Boeters (1988) puede ejemplificarse con los casos de los géneros *Horatia* Bourguignat, 1887 y *Neohoratia* Schütt, 1961, ya que en dicho trabajo se describen o citan nueve especies en esos dos géneros, que en realidad representan 19 especies y nueve géneros (Arconada y Ramos, 2003). Además, todas las especies ibéricas que Boeters (1988) asignó a *Horatia* o *Neohoratia*, pertenecen a otros géneros, lo que lleva a considerar que ninguno de estos géneros tienen representantes en la región íbero-balear (Arconada y Ramos, 2003).

En los trabajos más recientes publicados sobre la familia Hydrobiidae en la región íbero-balear se consideran 20 géneros y 64 especies descritas para este área (Boeters, 1988; Arconada, 2000; Ramos *et al.*, 2000; Arconada y Ramos, 2001, 2002, 2003, 2006, 2007a, 2007b; Boeters, 2003; Altaba, 2007; Arconada *et al.*, 2007a, 2007b; Glöer y Zettler, 2007; Arconada *et al.*, 2008; Alba *et al.*, 2009; Rolán y Oliveira, 2009; Rolán *et al.*, 2009; Rolán y Pardo, 2011) (ver Figura 2). De acuerdo con la hipótesis filogenética publicada con el objetivo de estudiar si el origen de la familia Hydrobiidae es monofilético o no (Wilke *et al.*, 2001), los

siguientes géneros quedarían excluidos por pertenecer a otras familias: *Moitessieria* y *Bythiospeum* (Moitessieriidae), *Bithynia* (Bithyniidae), *Semisalsa* (Cochliopidae) y *Bythinella* (Amnicolidae). Tampoco está incluido el género *Potamopyrgus* Stimpson, 1865 en las cifras aportadas, ya que la única especie que habita en la península Ibérica, *Potamopyrgus antipodarum* (Gray, 1843), es una especie invasora procedente de Nueva Zelanda que invadió Europa a mediados del siglo XIX (Gofas, 2012). Asimismo, los estudios filogenéticos que se están realizando actualmente sobre la superfamilia Rissooidea sugieren que los taxones de Nueva Zelanda probablemente pertenecen a otra familia diferente a Hydrobiidae (Haase *et al.*, 2007a).

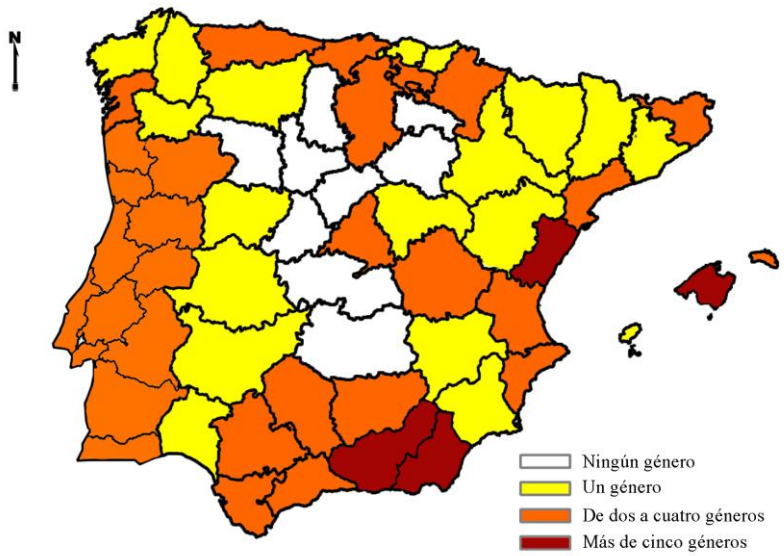

Figura 2. Mapa de abundancia de los géneros de hidróbidos por provincia de la región íbero-balear. Los datos se han obtenido a partir publicaciones previas (Boeters, 1988; Arconada, 2000; Ramos *et al.*, 2000; Arconada y Ramos, 2001, 2002, 2003, 2006, 2007a, 2007b; Boeters, 2003; Altaba, 2007; Arconada *et al.*, 2007a, 2007b; Glöer y Zettler, 2007; Arconada *et al.*, 2008; Alba *et al.*, 2009; Rolán y Oliveira, 2009; Rolán *et al.*, 2009; Rolán y Pardo, 2011).

Las revisiones taxonómicas recientes sobre los hidróbidos valvatiformes de la península Ibérica (Ramos *et al.*, 2000; Arconada y Ramos, 2001, 2006; Arconada *et al.*, 2007a; Arconada *et al.*, 2007b) han revelado que, además de las características de la concha, los sistemas genitales masculino y femenino y el sistema nervioso, otras estructuras anatómicas como los órganos paleales, el opérculo y las características generales del cuerpo podrían ser utilizados como caracteres taxonómicos para la identificación de especies de hidróbidos, así como para estudiar la variabilidad intraespecífica e interespecífica. Algunos de estos caracteres han sido también destacados como diagnósticos por Davis *et al.* (1976) y

han servido para diferenciar especies de otras regiones, como Australia (Ponder *et al.*, 1989) o Norteamérica (Hershler, 1994).

EL GÉNERO *PSEUDAMNICOLA* PAULUCCI, 1878

El género *Pseudamnicola* reúne un conjunto de especies de gasterópodos dulceacuícolas de sexos separados pertenecientes a la familia Hydrobiidae. La mayoría habita en fuentes, acequias y pequeños cursos de aguas permanentes, pero también han sido encontrados en lagos y ríos. *Pseudamnicola* fue propuesto por Paulucci (1878) para diferenciar a las especies europeas del género *Amnicola* Gould y Haldeman, 1840 de las americanas. Morfológicamente, las especies europeas se distinguen por presentar, según Paulucci (1878), una concha más pequeña, con escaso número de vueltas de espira y una vuelta del cuerpo más grande y voluminosa que el resto. Por su parte, la concha de las especies americanas tiene forma turbinoide, tienen el ombligo más marcado, las vueltas muy convexas y una apertura redondeada (Tryon, 1870). No obstante, Paulucci no designó una especie tipo para el nuevo género y fueron Kennard y Woodward (1926) quienes designaron posteriormente a *Bythinia lucensis* Issel, 1866 como especie tipo de *Pseudamnicola*.

Además de en Europa, *Pseudamnicola* ha sido también citado en el norte de África (Forbes, 1838; Pallary, 1926; Boeters, 1976; Ghamizi *et al*., 1997; Glöer *et al.*, 2010), este de Anatolia (Schütt y Sesen, 1993), China (Yu y Wang, 1977; Guo *et al.*, 1982; Yu y Zhang, 1982; Yu, 1987), Israel (Tchernov, 1971), Georgia (Badzoshvili, 1979), Tajikistán (Izzatullaev, 1973) y oeste de Turquía (Schütt y Bilgin, 1970). Sin embargo, muchas de estas citas requieren una confirmación de su estatus taxonómico, ya que la mayor parte de los caracteres anatómicos y morfológicos no están plenamente estudiados.

En Europa, *Pseudamnicola* se encuentra principalmente en las islas y países mediterráneos (Fauna Europaea, 2011), aunque se han citado algunas especies en el norte de Europa (Ehrmann, 1933; Adam, 1940; Fretter y Graham, 1978). La península Ibérica es una de las regiones más diversas en cuanto a hidróbidos se refiere (Arconada y Ramos, 2003), aunque todavía se requiere una revisión más profunda de la familia en este área. En relación al género *Pseudamnicola*, se han descrito 13 especies en la región íbero-balear (Paladilhe, 1869; Fagot, 1907; Altimira, 1960; Boeters, 1970, 1971, 1981, 1984, 1986, 1988, 1999). No obstante, la mayoría de estas descripciones son incompletas, ya que están basadas en un escaso número de caracteres, principalmente conquiliológicos.

Actualmente, se reconocen dos subgéneros dentro del género *Pseudamnicola*: *P. (Pseudamnicola)*, distribuido ampliamente a lo largo de la cuenca mediterránea, y *P. (Corrosella)*, encontrado únicamente en la península Ibérica y en unas pocas localidades del sur de Francia. Boeters (1970) describió el género *Corrosella* para un grupo de especies ibéricas cuyas conchas estaban erosionadas (corroídas, según Boeters), principalmente en su ápice. Anatómicamente, Boeters (1970) diferenció al nuevo grupo del resto de especies de *Pseudamnicola* por presentar una "bursa copulatrix"plegada, que es al menos dos veces más larga que la glándula del albumen, mientras que en *Pseudamnicola* es ligeramente más larga o incluso más pequeña en algunas especies. Años más tarde, este autor estableció *Corrosella* como un subgénero dentro de *Pseudamnicola* por no encontrar caracteres diagnósticos válidos y suficientes que dotaran a *Corrosella* de la categoría de género (Boeters, 1984).

En diversos trabajos, Boeters (1970, 1981, 1984, 1986, 1999) describió un total de siete especies del género *Pseudamnicola* en la región íbero-balear (ver Tabla 3 y Figura 3). En 1988, este mismo autor publicó una revisión monográfica sobre las especies de Hydrobiidae en la región íbero-balear en la que definía el área de distribución del género *Pseudamnicola* como la mitad este de la península Ibérica y las islas Baleares (Mallorca, Menorca e Ibiza). La última especie de este género descrita por Boeters es *P. (C.) hydrobiopsis* Boeters, 1999, encontrada en el sur de la península Ibérica. Todas sus descripciones están basadas en caracteres conquiliológicos y de los sistemas genitales masculino y femenino, y encontró altos rangos de variabilidad morfológica dentro de las especies. Una de las especies que mayor variabilidad intraespecífica y mayor área de distribución presentaba era *P. (P.) spirata* (Paladilhe, 1869) [actualmente *P. (P.) subproducta*, según Soler *et al.*, 2006], ya que se encontró en varios enclaves de la península Ibérica y en las islas Baleares. Glöer y Zettler (2007) y Altaba (2007) describieron cuatro especies nuevas de *Pseudamnicola* en las islas Baleares (Tabla 3; Figura 3), coincidiendo con algunas localidades de *P. (P.) subproducta*. En la presente tesis se revisarán todas las especies de *Pseudamnicola* descritas hasta el momento en la región íbero-balear, aplicando el concepto filogenético de especie para verificar el estatus taxonómico de cada una de ellas y establecer los límites interespecíficos e intraespecíficos.

Todas las especies del género *Pseudamnicola* descritas en el área objeto de estudio son especies endémicas de la región íbero-balear, excepto tres de ellas. La primera es *P. (P.) conovula* (Frauenfeld, 1863), cuya localidad tipo se encuentra en la isla de Pag (Croacia), que además ha sido citada en Túnez (Boeters, 1976) y Córcega, y, dentro de la región íbero-balear, en la provincia de Castellón (Gasull, 1981), el litoral catalán, Mallorca y Menorca (comunicación personal de Boeters a Gasull en Gasull, 1981). Sin embargo, en la monografía publicada años más tarde por el mismo Boeters (Boeters, 1988) la especie encontrada en el litoral catalán,

Mallorca y Menorca es la segunda especie no endémica de la región íbero-balear, *P. (P.) subproducta*. Además de en esas regiones, esta última especie ha sido citada en Marruecos (Ghamizi *et al.*, 1997), aunque los propios autores dicen no estar seguros de su presencia en este país.

Por último, *P. (C.) astieri* (Dupuy, 1851) fue originalmente descrita en los alrededores de Grasse, en el Departamento de Alpes-Maritimes (Francia). Otras especies procedentes del vecino departamento del Var (*Bythinella anteisensis* Berenguier, 1882, *B. berenguieri* Bourguignat en Berenguier, 1882, *B. doumeti* Bourguignat en Locard 1893, entre otras) han sido posteriormente sinonimizadas con *P. (C.) astieri* (en Falkner *et al.*, 2002 y Girardi, 2009). Gasull (1981) citó la presencia de *P. (C.) astieri* en la provincia de Castellón (España). Sin embargo, Falkner *et al.* (2002) confirmaron años más tarde que existieron ciertos malentendidos en el intercambio de información entre Boeters y Gasull (Boeters, comunicación personal), lo que llevó a pensar que esta especie habitaba en varias provincias del centro y del este de España (Gasull, 1981; Vidal-Abarca y Suárez, 1985). Como resultado, en el Catálogo de los moluscos continentales en Francia (Falkner *et al.*, 2002) se catalogó a *P. (C.) astieri* como endémica del departamento francés del Var. Por lo tanto, se estudiarán las localidades ibéricas asignadas en un principio a este especie para confirmar o no la hipótesis de Falkner *et al*. (2002).

A pesar de ser uno de los géneros más abundantes de la familia Hydrobiidae, son escasos los estudios filogenéticos que incluyen al género *Pseudamnicola*. El primer trabajo donde *Pseudamnicola* fue analizado y comparado genéticamente con otros géneros fue el de Wilke *et al.* (2001). Con el objetivo de estudiar el origen filogenético de la familia Hydrobiidae, en dicho trabajo así como en otro publicado más recientemente (Wilke *et al.*, 2013), se estudiaron varios géneros de Rissooidea y emplearon la especie tipo, *P. (P.) lucensis*, como representante del género *Pseudamnicola*. Así, los resultados finales en ambos estudios establecieron que *Pseudamnicola* se encontraba dentro de la subfamilia Pseudamnicolinae, junto con su grupo hermano, el género *Adrioinsulana* Radoman, 1978, y que las subfamilias Hydrobiinae y Pyrgulinae formaban a su vez el clado filogenéticamente más cercano (Figura 4). No obstante, años más tarde, en un artículo de revisión del género *Pseudamnicola* en los Balcanes, Szarowska *et al.* (2006) demostraron que *Adrioinsulana* no es un género independiente, sino un sinónimo posterior de *Pseudamnicola*. Este hecho, además del parentesco evolutivo existente entre *Pseudamnicola* con los géneros de las subfamilias Hydrobiinae y Pyrgulinae, fue confirmado en un estudio sistemático publicado en el mismo año (Szarowska, 2006) sobre los moluscos de la superfamilia Rissooidea en los Balcanes.

Tabla 3. Especies de *Pseudamnicola (Corrosella)* y *Pseudamnicola (Pseudamnicola)*, descritas previamente en la región íbero-balear y objeto de revisión en este trabajo.

Especie	Referencia	Especie	Referencia
P. (C.) astieri	Dupuy, 1851	*P. (P.) artanensis*	Altaba, 2007
P. (C.) falkneri	Boeters, 1970	*P. (P.) beckmanni*	Glöer y Zettler, 2007
P. (C.) hinzi	Boeters, 1986	*P. (P.) gasulli*	Boeters, 1981
P. (C.) hydrobiopsis	Boeters, 1999	*P. (P.) granjaensis*	Glöer y Zettler, 2007
P. (C.) luisi	Boeters, 1984	*P. (P.) meloussensis*	Altaba, 2007
P. (C.) navasiana	Fagot, 1907	*P. (P.) subproducta*	Paladilhe, 1869
		P. (P.) conovula	(Frauenfeld, 1863)

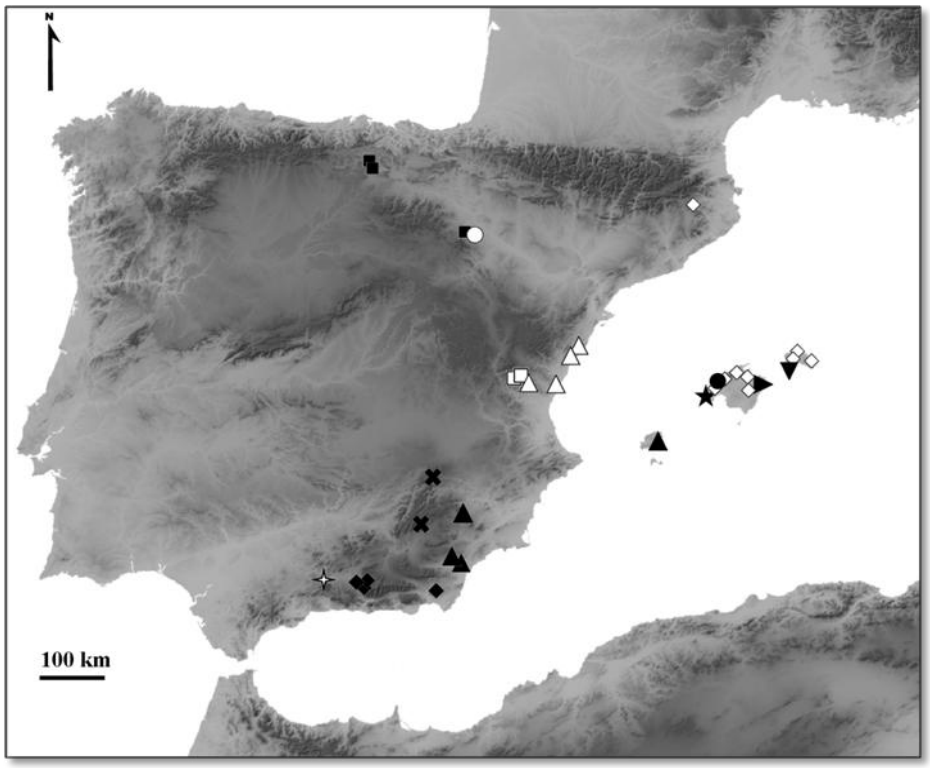

Figura 3. Mapa de distribución de las especies de *Pseudamnicola* descritas hasta el comienzo de la presente tesis, las cuales son objeto de revisión. Simbología mostrada en Tabla 3.

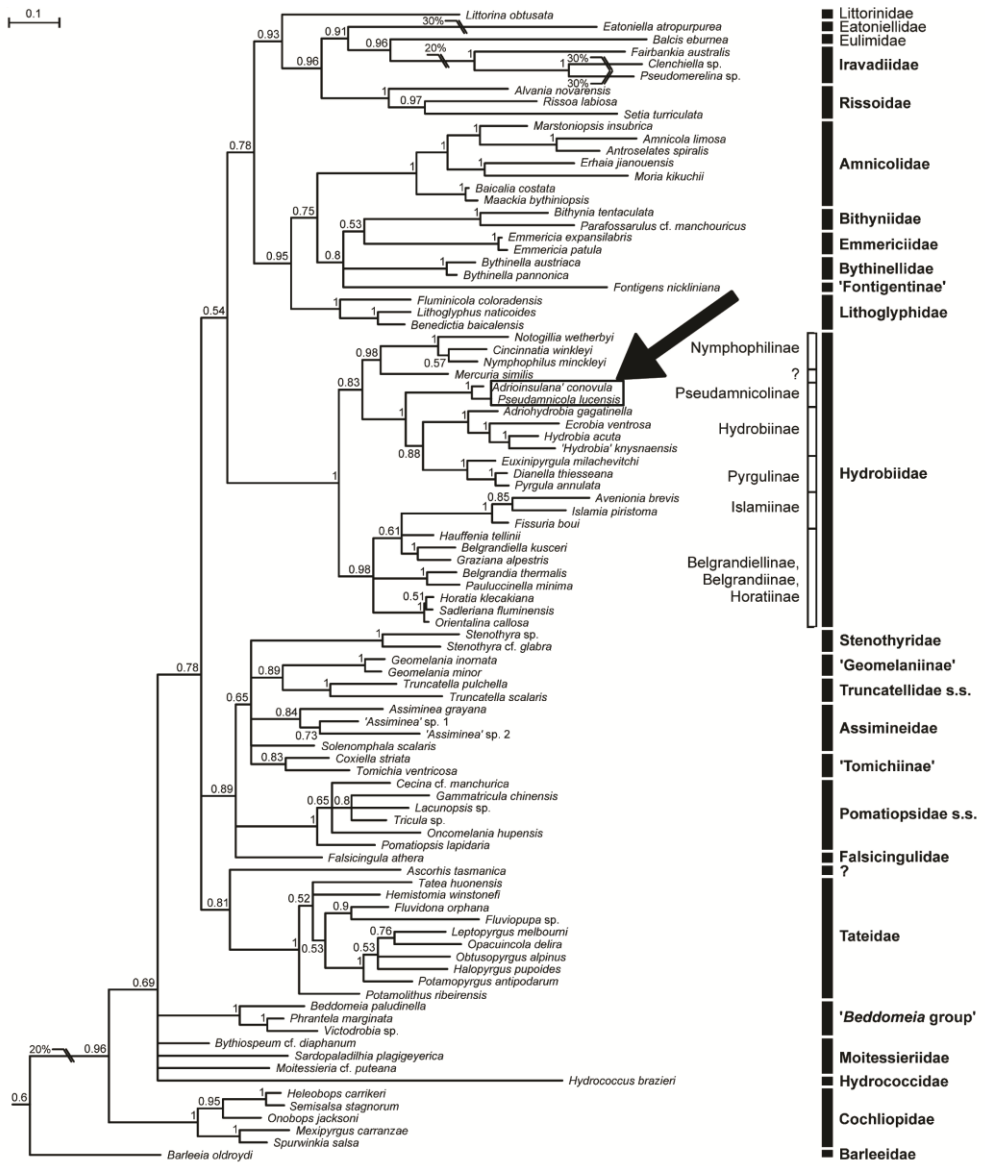

Figura 4. Filogenia obtenida por inferencia bayesiana para la superfamilia Rissooidea en el trabajo de Wilke *et al.* (2013), basado en la combinación de secuencias de los genes COI, 16S y 18S. Los valores sobre las ramas muestran las probabilidades posteriores para cada una de ellas y la flecha indica la posición de *Pseudamnicola* en la filogenia. Los nombres de las posibles familias y subfamilias dentro de Hydrobiidae se indican en el margen derecho.

El objetivo principal de esta tesis es el de progresar en el conocimiento de la diversidad biológica de los moluscos de agua dulce, en este caso del género *Pseudamnicola*, y, además, analizar las relaciones filogenéticas existentes entre sus especies. Para ello, se ha llevado a cabo una doble aproximación metodológica donde, por un lado, se revisa la clasificación taxonómica mediante un estudio

anatómico y morfológico de cada una de las especies encontradas en la región íbero-balear y, por otro lado, a través de filogenias moleculares se pretende apoyar genéticamente la validez de esas especies y estudiar el parentesco evolutivo existente entre ellas. Aplicando cada una de estas dos aproximaciones a cada uno de los dos subgéneros por separado, posteriormente se estudian conjuntamente ambos grupos para tener una visión global de la sistemática de *Pseudamnicola*. Asimismo, y con la finalidad de profundizar en el conocimiento de la historia evolutiva del grupo, se incluyen en los análisis filogenéticos algunas poblaciones adicionales procedentes de otros países e islas de la cuenca mediterránea.

HIPÓTESIS Y OBJETIVOS

A continuación, se exponen las hipótesis de partida a investigar en este trabajo y los objetivos propuestos para tal fin.

- **Hipótesis 1:** De acuerdo con los trabajos publicados por Boeters (1970, 1984, 1988), el género *Pseudamnicola* está compuesto por dos subgéneros, el nominotípico, *P. (Pseudamnicola)*, y *P. (Corrosella)*, cuya diferenciación está basada principalmente en caracteres conquiliológicos y del aparato genital femenino.

    - Objetivo. Para comprobar esta hipótesis, se estudiarán primero cada uno de los dos subgéneros en el área íbero-balear y después se analizarán conjuntamente. Tras la combinación de los datos, se examinarán los caracteres homólogos pertinentes de cada subgénero, comparándose los resultados obtenidos tanto en el enfoque morfológico como en el molecular. Además, se incluirán en los análisis filogenéticos poblaciones de otras regiones de la cuenca mediterránea, a fin de conseguir un acercamiento más completo a la sistemática y a la historia evolutiva del género *Pseudamnicola*.

- **Hipótesis 2:** El conjunto de especies del género *Pseudamnicola* que habitan en la región íbero-balear está formado por todas las descritas hasta la fecha. Todas ellas son endémicas de esta región, con excepción de *P. (C.) astieri* y *P. (P.) conovula*.

    - Objetivo. Para confirmarlo, primero se deberán visitar las localidades tipo de las especies previamente descritas y analizar muestras de sus ejemplares. Una vez confirmados o establecidos los caracteres diagnósticos de cada especie, se realizará un estudio comparativo morfológico y molecular de todas las poblaciones muestreadas o de la información existente en distintas bases de datos para su posterior clasificación en esas o en nuevas especies.

- **Hipótesis 3:** En relación a los estudios morfológicos, además de las características de la concha, los aparatos genitales masculino y femenino y el sistema nervioso, otras estructuras anatómicas como los órganos paleales, el opérculo, el sistema digestivo, la rádula y las características generales del cuerpo deben ser utilizados como caracteres descriptivos, e incluso diagnósticos de especies.

    - Objetivo. Para comprobar esta hipótesis se estudiarán los caracteres anatómicos comúnmente analizados en estudios taxonómicos de hidróbidos, junto con otros nuevos o menos utilizados. De esta manera, los nuevos resultados cualitativos y cuantitativos obtenidos serán

utilizados para la redescripción de especies previamente conocidas, y así poder establecer los límites intra e interespecíficos imprescindibles para identificar correctamente las especies o detectar otras nuevas para la Ciencia.

- **Hipótesis 4:** El tamaño pequeño o diminuto y la simplicidad de las estructuras de estos gasterópodos dificulta el reconocimiento de sus especies y, por lo tanto, su diversidad específica podría estar subestimada. Por esta razón, es necesario e incluso imprescindible el empleo de marcadores moleculares en combinación con estudios taxonómicos para la delimitación de esas especies y el conocimiento de sus relaciones filogenéticas.

    - Objetivo. Para apoyar genéticamente la validez de las "morfoespecies" y estudiar la variabilidad inter e intraespecífica de las poblaciones, se emplearán los marcadores moleculares adecuados para los hidróbidos. Además, a través del estudio de estos genes, se examinarán las relaciones filogenéticas existentes entre las especies de *Pseudamnicola*.

- **Hipótesis 5:** Todas las especies descritas para las islas Baleares pertenecen al subgénero *Pseudamnicola*. Asimismo, su presencia en las islas es consecuencia de fenómenos de vicarianza producidos por la separación de las mismas del continente durante el Mioceno inferior (aproximadamente hace 23 millones de años).

    - Objetivo. Para analizar esta cuestión, primero se comprobará tanto morfológica como genéticamente la inclusión de estas especies dentro del subgénero *Pseudamnicola*. Una vez conocidas sus relaciones filogenéticas con las especies peninsulares, se datará el origen de la diversificación a través de métodos de coalescencia asumiendo un reloj molecular.

- **Hipótesis 6:** Dada la escasa capacidad de dispersión que caracteriza a los hidróbidos, se postula que el principal mecanismo de especiación en este grupo es la fragmentación y el posterior aislamiento de sus poblaciones. Por lo tanto, la elevada actividad tectónica de la península Ibérica, junto con los cambios climáticos acontecidos durante su historia geológica, deben haber tenido un papel crucial sobre los patrones evolutivos encontrados para este género.

    - Objetivo. Una vez definidos los taxones ibéricos, se aplicarán técnicas de datación molecular para verificar si la formación de esas barreras geográficas o las fluctuaciones climáticas datadas hasta el momento, han producido fenómenos de especiación alopátrica que definian los patrones evolutivos de *Pseudamnicola*.

MATERIAL Y MÉTODOS

ÁREA DE MUESTREO Y MATERIAL DE ESTUDIO

El área geográfica muestreada en el presente estudio ha sido principalmente la península Ibérica, las islas Baleares y el Departamento de Var (sur de Francia). Además, se han estudiado otras poblaciones del género *Pseudamnicola* procedentes de Cerdeña, Sicilia, Italia continental, Grecia y Túnez (Figura 5). El material recolectado y estudiado ha sido fruto de un trabajo colectivo en el que han participado otros investigadores del Museo Nacional de Ciencias Naturales (MNCN) y de otras instituciones, que realizaron diversas campañas de muestreo en ambientes dulceacuícolas desde 1982. Como resultado de ello, sólo en la región íbero-balear se ha contado con material de moluscos dulceacuícolas de alrededor de 1.500 localidades y, de este total, aproximadamente 150 localidades contenían las poblaciones del género *Pseudamnicola* estudiadas en esta tesis.

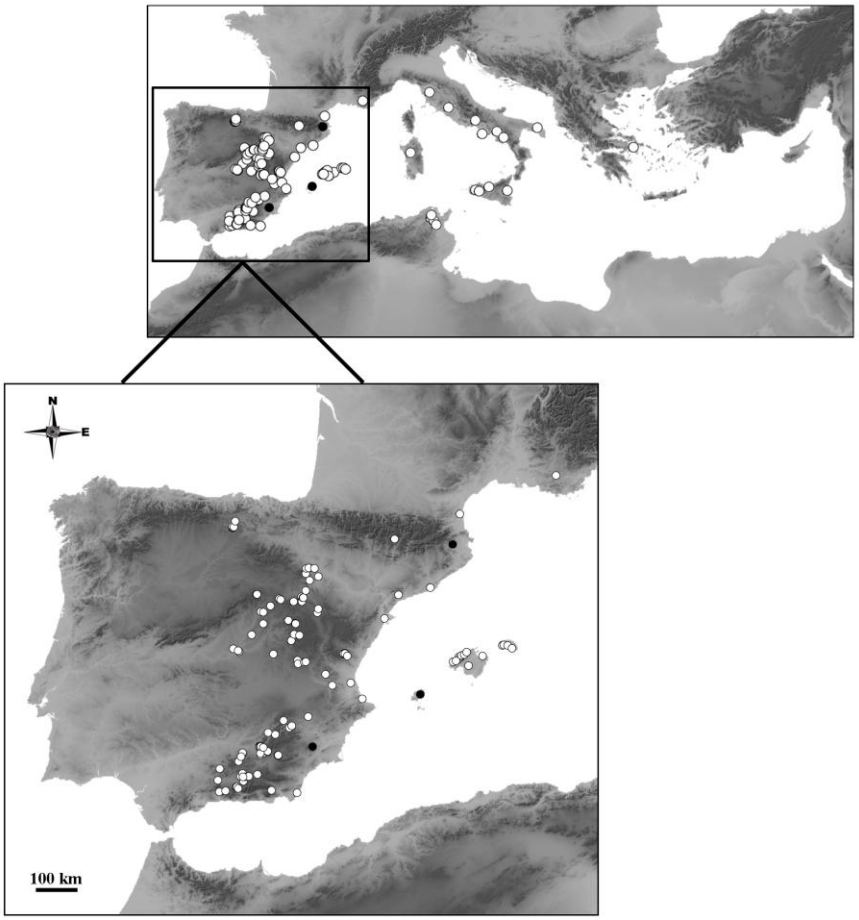

Figura 5. Mapa de distribución de las poblaciones estudiadas, con detalle de las localidades íbero-baleares. Los puntos negros (en esta figura y en el resto de mapas) ilustran localidades reseñadas en referencias bibliográficas que se han vuelto a visitar y en las que no se ha encontrado de nuevo la especie citada.

Uno de los grandes problemas en los estudios sistemáticos es que, al igual que en otras disciplinas, la validez de los resultados depende en gran medida del diseño del muestreo. Por esta razón, es fundamental encontrar un equilibrio entre el muestreo de las poblaciones y el área geográfica objeto de estudio. Debido a que el género *Pseudamnicola* tiene una amplia distribución circunmediterránea, es complicado, aunque no imposible, realizar un muestro completo en toda el área y caracterizar adecuadamente todas o la mayoría de las poblaciones durante el tiempo habitual de desarrollo de una tesis doctoral. Por ello, el presente trabajo ha dado prioridad a la región íbero-balear, ya que, por una parte, es una de las áreas con mayor riqueza de hidróbidos (un punto caliente de diversidad junto a las penínsulas balcánica e itálica, según Davis, 1982), y, por otra, el género *Pseudamnicola*, que necesita una profunda revisión, tiene una importante representación en la región. Además, esta tesis se enmarca en el proyecto Fauna Ibérica. El estudio y el muestreo se han planteado como sigue:

1) Prospección en las localidades tipo de los taxones descritos y otras localidades citadas en la bibliografía.

2) Nuevas localidades encontradas tanto en las proximidades de las localidades tipo como en otras regiones.

3) Estudio del material colectado previamente en el marco del proyecto Fauna Ibérica y en otras campañas, depositado en el Museo Nacional de Ciencias Naturales (listado de colectores en Tabla 4).

4) Estudio del material tipo depositado en colecciones de otros museos y universidades europeas (Tabla 5).

Tabla 4. Listado de abreviaturas y nombres correspondientes a los colectores de las muestras estudiadas en el presente trabajo. La relación de los colectores con las muestras se muestra en el Apéndice II.

B.A.	Beatriz Arconada	J.Q.	Josep Quintana
C.N.	Carolina Noreña	M.A. R.	Marian Ramos
D.D.	Diana Delicado	M.C.	Marta Cortés
D.M.	Diego Moreno	M.V.	Miquel Vila
E.R.	Emilio Rolán	N.M.	Nuria Martín
I.B.	Irene Ballesta	O.S.	Óscar Soriano
J.B.	Jaime Bosch	R.A.	Rafael Araujo
J.M. R.	José Miguel Remón	R.M. A.	Ramón Manuel Álvarez
J.M. B.	José Miguel Barea	S.J.	Silvia Jiménez

Además, para completar el análisis lo máximo posible y obtener una aproximación a la historia evolutiva de este grupo de gasterópodos, se han incluido en el trabajo 24 poblaciones del sur de Francia, Cerdeña, Sicilia, Italia continental, Túnez y Grecia (Figura 5 y Tabla 13), como se ha indicado anteriormente. Las recolecciones se realizaron durante todas las épocas del año. El Apéndice II contiene una relación de todas las localidades íbero-baleares incluidas en este trabajo.

Los tipos de medio donde se han encontrado poblaciones del género han sido principalmente manantiales, fuentes con caño, acequias, ojos ("ullals"), arroyos, balsas, lagunas e incluso tramos de río de escasa corriente (Figura 6). Todos estos medios tienen aguas permanentes y con un flujo lento, lo que hace que permanezcan limpias y oxigenadas. Estos hidróbidos requieren hábitats con aguas limpias y con suficiente vegetación y materia orgánica para alimentarse.

Figura 6. Tipos de hábitats. **A.** Manantial Pozo Azul, Burgos. **B:** Source d'Argens, Brue-Aurillac, Var, Francia. **C.** Acequia en la Ermita de Betlem, Mallorca. **D.** Fuente Pilar del Mono, Dúrcal, Granada. **E.** "Ullal" de Baltasar, Amposta, Tarragona. **F.** Lago de Bañolas, Gerona.

Tabla 5. Abreviaturas de las colecciones citadas en este estudio.

BOE	Colección personal de H. D. Boeters
CRA	Colección personal de C. Altaba
FALK	Colección personal de G. Falkner
GAS	Colección personal de L. Gasull
MNCN	Museo Nacional de Ciencias Naturales, CSIC, Madrid
MNHN	Muséum National d'Histoire Naturelle, Paris
NHMW	Naturhistorisches Museum Wien, Vienna
NNM	Natural History Museum Naturalis, Leiden Anteriormente: RMNH, Rijksmuseum van Natuurlijke Historie
SMF	Forschungsinstitut und Natur-Museum Senckenberg, Frankfurt
ZMH	Zoologischen Museum, Hamburg

TÉCNICAS DE MUESTREO

La técnica de recolección dependió del substrato donde habitaban los ejemplares. Si se encontraban adheridos a las piedras, se extraían directamente uno a uno con pinzas blandas. Si, por el contrario, estaban en el sedimento del fondo, se tamizaba éste con un colador y se volcaba su contenido sobre una bandeja, preferiblemente de color blanco para un mayor contraste, y, con la ayuda de pinzas blandas, se recolectaban los individuos. En el caso de que habitaran en la vegetación u hojarasca, el procedimiento llevado a cabo fue lavar ésta manualmente en una bandeja blanca con el fin de despegar los ejemplares. Si se encontraban sobre las paredes de las fuentes éstas se barrían manualmente o con un pincel, situando inmediatamente debajo una bandeja para recolectar el material. Y, por último, en una de las localidades, la localidad tipo de la especie *P. (C.) hydrobiosis* (fuente La Carmonilla, Loja, Granada), se aplicaron todo tipo de métodos, incluyendo el de bombeo de Bou-Rouch (Bou y Rouch, 1967), técnica empleada para extraer ejemplares de las aguas intersticiales, y que es el que se menciona en la descripción original de esta especie (Boeters, 1999).

En cada una de las localidades de muestreo se completó una ficha de recolección, en la que se anotaron todos los datos correspondientes a la localidad: descripción, acceso, coordenadas geográficas, parámetros físico-químicos, etc. En cada punto de muestreo se tomaron las coordenadas X/Y del sistema UTM ("Universal Transverse Mercator") con un GPS eTrex Legend Cx (Garmin). En

aquellas localidades en las que no fue posible tomar las coordenadas *in situ*, éstas se obtuvieron a partir de los mapas del Servicio Geográfico del Ejército (SGE) a escala 1:50.000. La toma de datos de las condiciones físico-químicas del agua sólo se realizó en algunas localidades del muestreo. Las medidas tomadas fueron la temperatura (en °C) y la conductividad del agua (en µS y mS), medida con un conductivímetro CRISON CDTM-523.

PREPARACIÓN DEL MATERIAL

Después de la recolección, los ejemplares vivos se conservaron en una nevera y en el agua de la localidad de origen hasta llegar al laboratorio. Una vez allí, y dependiendo del objeto de su estudio, el material se separó para ser fijado siguiendo diferentes procesos.

Para los estudios morfológicos y anatómicos primero se observaron los individuos vivos con un estereomicroscopio Leica MZ16 A para tomar nota de los caracteres que pueden modificarse una vez fijado el material, como la pigmentación de la cabeza, la forma y pigmentación del pene, el pigmento y el tamaño de los tentáculos y el pie y la coloración de la concha. A la vez que se observaron, también se fotografiaron los ejemplares empleando una cámara Nikon Ds-Fi1 acoplada a dicho estereomicroscopio. Una vez tomados los datos preliminares, se anestesió una parte de los individuos añadiendo una pequeña cantidad de cristales de mentol en la superficie del agua de la localidad en la que se transportaron los ejemplares, con el fin de fijarlos relajados y así facilitar el estudio anatómico y morfométrico (ver Araujo *et al*., 1995). Se conservaron de 12 a 24 horas a 4 °C en una cámara fría, porque de esta manera el anestésico actúa más lentamente y permite un mayor control del estado de los ejemplares. A continuación, se extrajo el mentol, se volcó el contenido del bote en un colador y éste se sumergió unos segundos en agua caliente (entre 60-70 °C) para producir un choque térmico antes de fijar los ejemplares en etanol de 70%. De esta manera se evita que los individuos se retraigan y puedan quedar contraídos dentro de la concha en el caso de que estuvieran aún vivos al sumergirlos en el alcohol (Arconada, 2000).

Para los estudios moleculares se fijó otra parte del material en etanol absoluto, de 96% o se congeló a -80 °C. Antes de la fijación, y para favorecer que el tejido se embebiese correctamente en el alcohol, se procedió a hacer un pequeño agujero en la superficie de la concha empleando para ello un alfiler entomológico o una minucia, de forma que el ejemplar no quedase destruido por completo.

Todo el material recogido, después de ser procesado, se etiquetó y almacenó hasta su estudio, para ser finalmente depositado en la colección de Malacología y en la de Tejidos y ADN del MNCN.

ESTUDIOS ANATÓMICOS, MORFOLÓGICOS Y MORFOMÉTRICOS

Estudios anatómicos y morfológicos

Para llevar a cabo estos análisis, se diseccionaron un total de cuatro hembras y cuatro machos de la localidad tipo de cada especie (cuando se encontró suficiente material). En el resto de localidades, el número de ejemplares fue de uno o dos machos y una o dos hembras para confirmar la identidad de los ejemplares y obtener información de la variabilidad entre poblaciones.

Para la disección, toma de fotografías y de medidas se empleó un estereomicroscopio Leica MZ16 A con una cámara Nikon DS-Fi1 acoplada y el programa Nis-Elements v. 2.2. El programa Auto-Montage Essentials v. 5.00 se utilizó para la alineación en el eje Z de las fotografías seriadas de conchas. Las descripciones anatómicas se completaron con dibujos esquemáticos de animales fijados en etanol de 70°, realizados con las cámaras claras de un estereomicroscopio Zeiss Stemi SV 8 o del Leica MZ16 A.

Dado el pequeño tamaño de los ejemplares, al comienzo de cada disección trató de evitarse el riesgo de eliminar la concha rompiéndola simplemente con las pinzas, ya que todo el ejemplar podría ser destruido. Por esta razón, se sumergió a temperatura ambiente el ejemplar en una solución de EDTA (ácido etilendiaminotetracético) diluido al 5% en agua destilada. Este reactivo consigue eliminar los depósitos calcáreos de la concha, dejando sólo el periostraco y la matriz orgánica de ésta, que pueden ser fácilmente eliminados. El tiempo transcurrido varió entre 12 y 24 horas. Una vez eliminada la concha, se procedió a realizar la disección en agua en una placa Petri que contenía una mezcla de parafina, cera y carbón activo (Davis, 1967), lo que confiere un color negro al fondo que contrasta con las partes blandas de los ejemplares.

Caracteres morfológicos y anatómicos estudiados

Uno de los principales problemas al abordar un estudio riguroso de los caracteres anatómicos de los hidróbidos consiste en la heterogeneidad terminológica

empleada en las descripciones por los diferentes especialistas, lo que dificulta los estudios comparativos. Por esa razón, y con el fin de establecer estándares, Hershler y Ponder (1998) publicaron una revisión exhaustiva de todos, o de la mayoría, de los caracteres morfológicos (y sus estados) observables en la familia Hydrobiidae *s. l.* Las descripciones morfológicas realizadas en esta tesis siguen de la forma más rigurosa posible dicha terminología, excepto para aquellos caracteres del sistema nervioso no incluidos en dicha revisión, cuyas caracterización ha sido basada en los trabajos de Davis y colaboradores (1976, 1984, 1986, 1992). Algunas estructuras glandulares, como los testículos o los ovarios no han sido estudiadas con detalle, puesto que el tipo de fijación y la técnica de estudio (disecciones bajo estereomicroscopio binocular) no mostraron suficiente resolución para estudiar dichos órganos. Por otro lado, este trabajo ofrece un estudio detallado de los siguientes órganos y sistemas (esquematizados en la Figura 7):

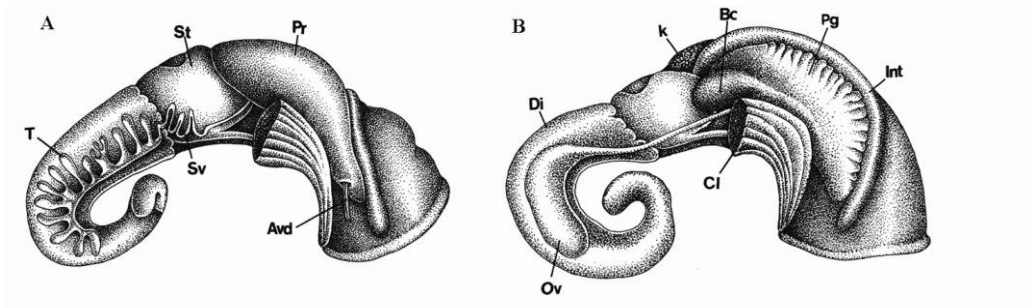

Figura 7. Esquema general del cuerpo de un hidróbido macho (**A**) y hembra (**B**), según Arconada y Ramos (2000). Avd: vaso deferente anterior; Bc: bolsa copuladora; CI: músculo columelar; Di: glándula digestiva; Int: intestino, k: riñón; Ov: ovario; Pg: oviducto paleal; Pr: próstata; St: estómago; Sv: vesícula seminal; T: testículos.

Concha. La concha de los hidróbidos es generalmente pequeña (1,5 a 6 mm), aunque su longitud varía según el género; es lisa, de vueltas convexas y de formas diversas (descritas en Hershler y Ponder, 1998). El número de vueltas de espira suele estar entre 4 y 6. Generalmente es transparente y la coloración la proporciona el periostraco, frecuentemente de color amarillo-translúcido. La concha está dividida en protoconcha y teleoconcha (Figura 8A). La concha embrionaria (protoconcha en *Pseudamnicola*), se forma durante el desarrollo embrionario y larvario del individuo y permanece visible e identificable en el ápice del ejemplar adulto. Presenta varios caracteres con valor taxonómico, entre ellos múltiples tipos de microescultura (Figura 8B). La teleoconcha empieza a formarse tras la eclosión del huevo y tiene una microescultura diferente, generalmente axial, formada por suaves líneas de crecimiento convexas, y termina en la abertura de la concha o peristoma, cuya forma y tamaño son caracteres diagnósticos de las especies. En el

adulto, la abertura es completa y la forma de su labio interno y externo varía entre las especies del género *Pseudamnicola*.

Opérculo. El opérculo de los hidróbidos es de naturaleza córnea y se encuentra en la parte dorsal del pie. Es generalmente ovalado o redondeado, blando, semitranslúcido, pauciespiral (es decir, con pocas vueltas de espira) y tiene un núcleo subcentral o central (Hershler y Ponder, 1998). El color está probablemente relacionado con su grosor; los opérculos más gruesos tienden a ser más opacos. En *Pseudamnicola* no existe ninguna protuberancia en el núcleo de la cara interna del opérculo, tal y como sucede en otros hidróbidos de los géneros *Hauffenia* y *Hemistomia* (Kuscer, 1932; Radoman, 1983), sino un ligero engrosamiento que sirve como zona de anclaje del músculo pedio. Esta región de contacto puede observarse en la cara interna del opérculo. La cara externa presenta líneas de crecimiento axial muy tenues (Figura 8C).

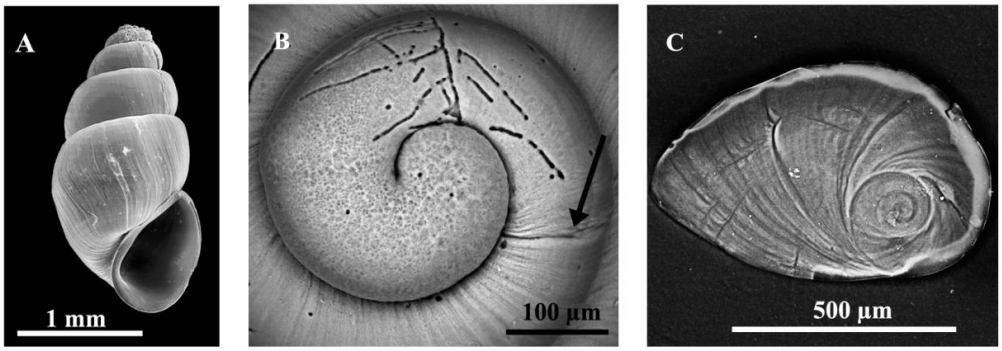

Figura 8. Microfotografías al ESEM de *Pseudamnicola (Corrosella) falkneri* de: **A.** Concha. **B.** Protoconcha. **C.** Opérculo. La flecha negra en B señala el límite de la protoconcha y el comienzo de la teleoconcha.

Cabeza-pie. Los hidróbidos presentan una cabeza y un pie bien desarrollados. La cabeza termina en un morro prominente y generalmente blanquecino; la forma y el tamaño de los lóbulos distales es variable entre especies. Los tentáculos cefálicos son típicamente alargados y estrechos y salen desde ambos lados de la cabeza. Cerca de su base se encuentran las regiones oculares (Figura 9). El pie es generalmente delgado y contiene una glándula mucosa anterior que vierte a través de una abertura transversal. La mayoría de los hidróbidos epigeos tienen pigmentación sobre la cabeza, los tentáculos, el pie y el epitelio externo de la cavidad paleal y de la vuelta visceral del cuerpo (especialmente en la zona dorsal). Muchos de estos caracteres se han observado en los ejemplares vivos, ya que los reactivos de fijación pueden contraer los órganos y alterar el color real de los mismos.

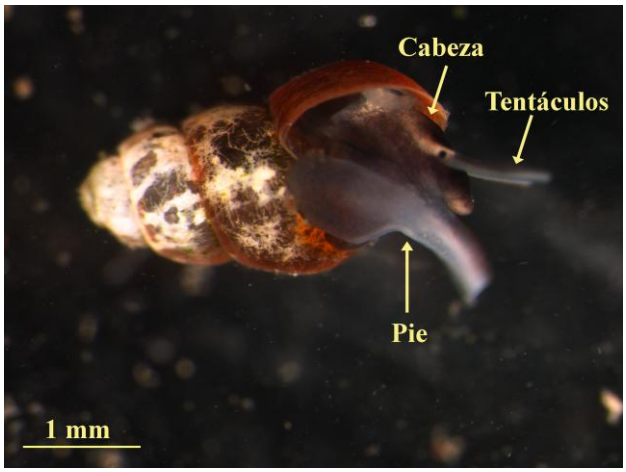

Figura 9. Fotografía de un ejemplar vivo de *Pseudamnicola (Corrosella) hinzi*.

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Cavidad paleal. La cavidad paleal de los hidróbidos está abierta al exterior y contiene órganos completos, como el osfradio y el ctenidio (Figura 10), y otros protegidos por un fino epitelio, como la próstata o el oviducto paleal, el pericardio, el recto y el ano. La intensidad de la pigmentación del epitelio externo del manto varía entre las diferentes especies. Las especies de *Pseudamnicola* generalmente tienen un ctenidio bien desarrollado que ocupa la mayoría de su superficie, un pequeño osfradio ventral al ctenidio, el gonoducto y el recto. El ctenidio es un órgano de función respiratoria que contiene una serie de laminillas branquiales (o lamelas) de forma triangular, fusionadas por su base al epitelio interno de la cavidad paleal, mientras que su ápice se encuentra libre para realizar el intercambio de gases. El osfradio es un órgano quimiosensorial.

Figura 10. Ctenidio y osfradio de *Pseudamnicola (Corrosella) hauffei*.

Sistema digestivo. Comienza en la boca, situada en la parte anterior del morro que contiene en su interior el bulbo bucal con la rádula. El bulbo bucal está conectado a un par de glándulas salivares de forma tubular y simple. El esófago es estrecho y no contiene glándulas esofágicas. A lo largo de su recorrido puede presentar uno o varios bucles en algunas especies, especialmente en el tramo posterior al sistema nervioso.

La rádula es un órgano quitinoso utilizado para raspar el alimento, que se halla dentro del bulbo bucal. El número de filas de dientes es muy variable entre las especies e incluso de unos individuos a otros. Su forma es ondulosa, generalmente en forma de "S", y su longitud puede ser pequeña (menor del 15% de la longitud total de la concha), mediana (entre el 25 y 30% de la longitud de la concha) o grande (mayor del 40% de la longitud de la concha). La zona anterior (o de destrucción) se caracteriza por tener los dientes gastados o rotos, mientras que en la zona posterior (o de crecimiento) los dientes están poco formados o aún incompletos. En los hidróbidos la rádula es típicamente tenioglosa, es decir, posee siete dientes por fila: uno central, dos laterales, dos marginales internos y dos marginales externos. Todos ellos contienen a su vez hileras de cúspides en sus bordes y, además, el diente central puede tener cúspides basales. El diente central (Figura 11A-1) es trapezoidal y, dentro de su fila de cúspides, destaca la central por ser de mayor tamaño. Su lengua basal, con forma de V o de U, puede incluso alcanzar la misma longitud que sus márgenes laterales. El diente lateral (Figura 11A-2) no presenta ninguna lengua basal y está compuesto por un ala lateral y una región rectangular, llamada cara del diente. Los dientes marginales (internos Figura 11A-3 y externos Figura 11A-4) no tienen ninguna cúspide basal, presentan unas alas laterales muy largas y un mayor número de cúspides en sus bordes, todas de tamaño semejante y uniformemente distribuidas.

El estómago se divide en dos partes: la más anterior, o saco del estilo, y la posterior, dividida a su vez en dos cámaras (Figura 11B). El saco del estilo tiene un epitelio monoestratificado con células ciliadas que empujan el alimento hacia la parte posterior del estómago. Las dos cámaras que constituyen la segunda porción del estómago presentan un tamaño relativo similar entre las diferentes especies. La cámara posterior se caracteriza por presentar una o dos entradas al hepatopáncreas o glándula digestiva; en algunos géneros aparece un ciego gástrico sobre el margen posterior de esta cámara. El intestino se inicia cerca del borde anterior del saco del estilo, e incluso algunas veces este último sobresale anteriormente al lazo que forma el intestino. El recto discurre dentro de la cavidad paleal y adopta varias formas, dependiendo del género de hidróbido; la más simple es la de U y la más compleja la de S, que es la que caracteriza al género *Pseudamnicola*.

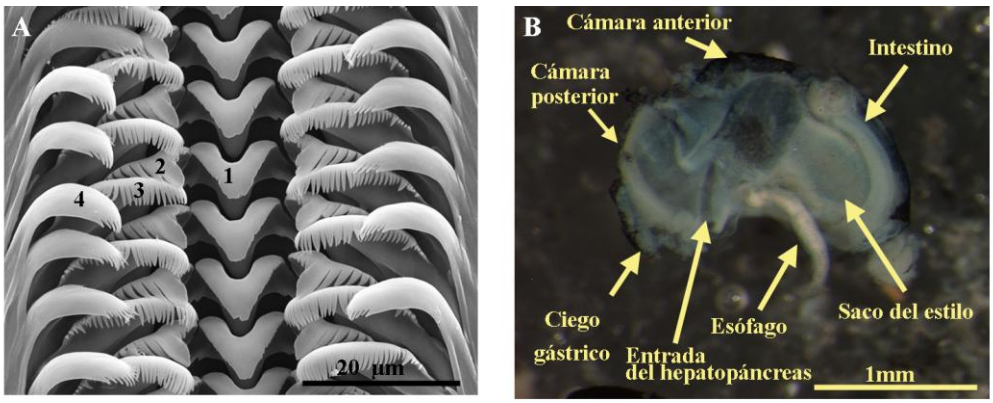


Figura 11. Detalle de la rádula al ESEM (**A**) y fotografía óptica de las partes del estómago (**B**) de *Pseudamnicola (Corrosella) falkneri*. Los números sobre la rádula indican los tipos de dientes: 1. Diente central. 2. Diente lateral. 3. Diente marginal interno. 4. Diente marginal externo.

Sistema genital masculino. Está compuesto principalmente por el testículo, la próstata y el pene. El testículo es similar en posición y extensión al ovario y al igual que éste, no llega a alcanzar la última región de la vuelta visceral. Ambos órganos genitales pueden ocupar hasta el 66% de la vuelta visceral y se sitúan inmediatamente detrás del estómago. El testículo está formado por una serie de lóbulos blanquecinos, simples (en alguna ocasión son de tipo compuesto) y alargados que vierten mediante estrechos vasos eferentes a un conducto mayor denominado vaso deferente. La glándula de la próstata es completa, cerrada, con forma de haba alargada, y su extremo posterior se encuentra dentro de la cavidad paleal. A su región media-posterior llega un conducto que procede de la vesícula seminal, y de su extremo anterior emerge el vaso deferente paleal que se dirige al pene (Figura 12A). La forma y el tamaño del pene varían de unas especies a otras. Siempre se inserta en el centro o en la mitad derecha de la cabeza, en la llamada "región morfogénetica del pene" (Le Gall, 1981). *Pseudamnicola* se caracteriza por poseer un pene simple, pigmentado y en su interior el conducto peneal recorre su longitud, recto u ondulado y pegado a la pared externa (Figura 12B). El pene también está sujeto a modificaciones una vez que se fijan los ejemplares, ya que su tejido es fundamentalmente muscular y puede contraerse. Por este motivo se ha estudiado también en ejemplares vivos.

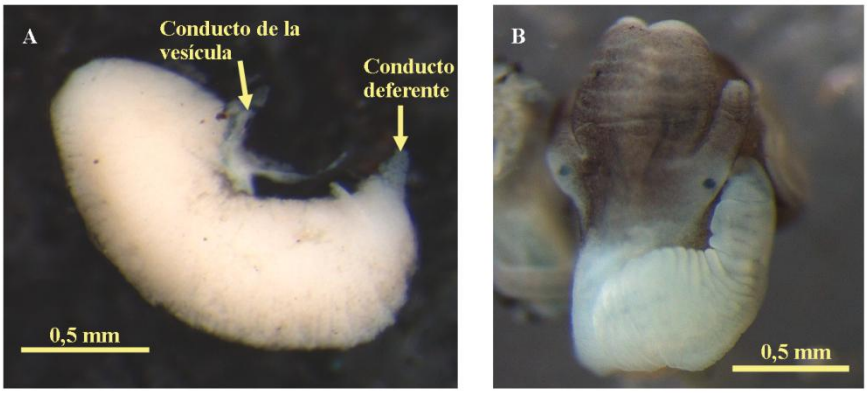


Figura 12. Fotografías de la próstata (**A**) y de la cabeza y el pene (**B**) de un macho de *Pseudamnicola (Pseudamnicola) beckmanni*.

Sistema genital femenino. Constituido por el ovario y la región anterior de la genitalia femenina (genitalia distal según Hershler y Ponder, 1998).

El ovario se encuentra en la parte posterior del cuerpo, detrás del estómago y dorsal a la glándula digestiva. Normalmente ocupa el 66% de la longitud total de la vuelta visceral.

La **genitalia distal** está formada por una serie de conductos, receptáculos y glándulas (Figura 13A), que proporcionan un amplio grupo de caracteres diagnósticos: La bolsa copuladora es un saco que tiene como función la acumulación y lisis de esperma del tipo "no orientado". En algunos géneros, como en *Tarraconia* Ramos y Arconada, 2000, se ha visto que su epitelio es de tipo columnar, aparentemente sin cilios y con espermatozoides desordenados en su interior (Ramos *et al.*, 2000). La bolsa copuladora se sitúa parcialmente por detrás de la pared de la cavidad paleal, y generalmente sobresale por encima de la cápsula del albumen. En su región anterodorsal se inserta el denominado conducto de la bolsa copuladora, generalmente largo en *Pseudamnicola*, cuya función es almacenar esperma de tipo "orientado" y conectar la bolsa copuladora con el canal ventral (Figura 13B). Éste es un canal abierto ventralmente, formado por pliegues del tejido de la cápsula glandular, que termina en el gonoporo. En este canal el macho introduce el esperma para la fecundación. El oviducto renal o libre es un conducto que transcurre ventralmente desde el ovario hasta su unión con la bolsa copuladora y el canal ventral. En esta región de unión transcurre en forma de uno o varios bucles. En *Pseudamnicola* y géneros afines, como *Hydrobia* o *Ecrobia*, el oviducto renal es pigmentado, a diferencia de lo que ocurre en la mayoría de hidróbidos. Su función es trasportar los oocitos desde el ovario hasta la genitalia distal. En su parte anterior, el oviducto renal se ramifica, dando lugar al conducto gonopericárdico, el cual se abre a la cavidad pericárdica y forma parte del riñón. Debido al pequeño diámetro de este

conducto, en las disecciones resulta muy difícil no romperlo, por lo que se observa en muy pocas ocasiones. El receptáculo seminal es un saco espermático que almacena el esperma de forma orientada y se origina en el oviducto renal, pudiendo tener un corto conducto (Ponder y Lindberg, 1997). Las glándulas del oviducto paleal están situadas en el lado derecho de la cavidad paleal, y son la glándula de la cápsula, más anterior, y la glándula del albumen. Esta última tiene como función nutrir al huevo en su paso por el oviducto paleal y se encuentra habitualmente en el interior del cuerpo. La glándula de la cápsula, cuya región anterior se ubica parcial o totalmente en la región paleal, es la encargada de producir la cubierta externa de huevo. En algunos individuos se ha observado una doble zonación dentro de esta cápsula. Aunque en este trabajo no se han realizado cortes histológicos, Hershler y Ponder (1998) encontraron una distinción entre los tejidos empleando colorantes tricrómicos.

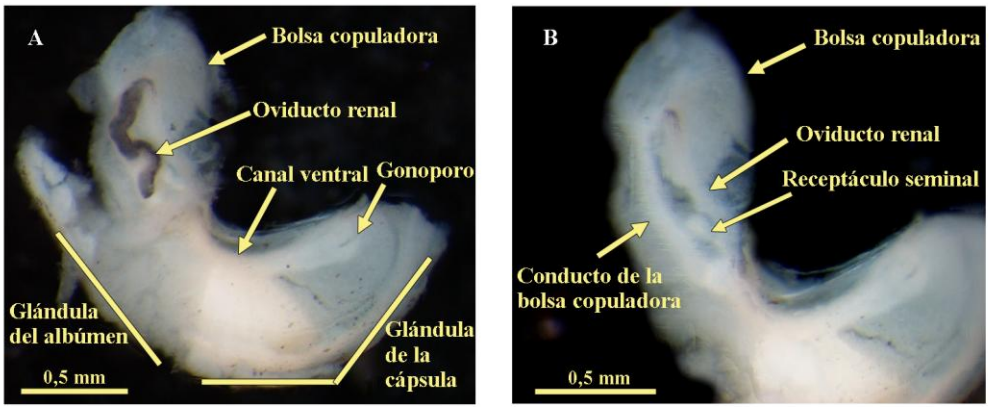


Figura 13. Fotografías de la genitalia distal de *Pseudamnicola (Corrosella) luisi* indicando cada una de sus partes. La imagen **A** muestra una visión general y la **B**, ya cortado el oviducto renal, muestra con más detalle su parte posterior.

Sistema nervioso. La parte principal del sistema nervioso se sitúa alrededor del bulbo bucal, en la región cefálica. En esta tesis se describe la parte dorsal del anillo nervioso de todas las especies estudiadas, que consta de una serie de ganglios, conectivos (conexiones entre pares de ganglios diferentes) y comisuras (conexión de un mismo par de ganglios). En la Figura 14 se muestran cada una de estas estructuras. En visión dorsal se observa la parte anterior del esófago, que pasa justo por debajo de la comisura cerebral, y un conjunto de diminutos ganglios que forman un "quiasma" entre sí y, debido a la torsión de los gasterópodos, dejan entre medias el conducto del esófago. Debido a su pequeño tamaño (de 30 a 40 µm), el sistema nervioso está muy poco estudiado y, sin embargo, los tamaños y las formas y, sobre todo, la proporción relativa entre las longitudes de estas estructuras pueden tener un carácter diagnóstico y evolutivo importante, pues proporcionan una medida

de la concentración ganglionar de la parte dorsal del anillo nervioso. Esta medida se estima empleando el índice "RPG" ("RPG ratio", Davis *et al*., 1976): longitud del conectivo supraesofágico dividida entre la suma de las longitudes del ganglio pleural derecho, del conectivo supraesofágico y del ganglio supraesofágico. El criterio adoptado en este trabajo sigue el establecido en varios estudios sobre hidróbidos (Davis *et al*., 1984, 1986, 1992): anillo nervioso dorsal concentrado (RPG ≤ 0,29), moderadamente concentrado (RPG = 0,30-0,49), alargado (RPG = 0,50-0,67) y extremadamente alargado (RPG ≥ 0,68).

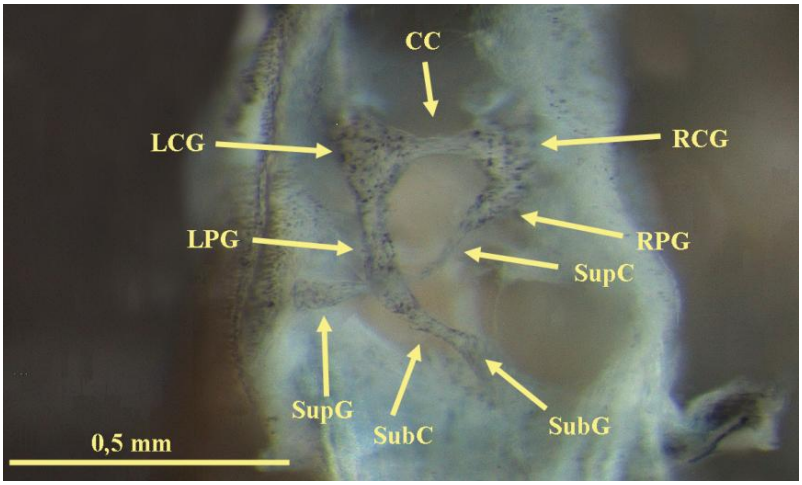


Figura 14. Imagen del anillo dorsal del sistema nervioso de *Pseudamnicola (Corrosella) navasiana* indicando cada estructura. Las abreviaturas están descritas en la Tabla 6.

Microscopía electrónica de barrido ambiental (ESEM)

El estudio de microscopía electrónica se realizó principalmente en ejemplares de las localidades tipo, tomando habitualmente de dos a cuatro conchas, tres rádulas y cuatro opérculos por población. Las observaciones y las microfotografías de conchas, rádulas y opérculos se realizaron con un microscopio electrónico de barrido ambiental (Environmental Scanning Electron Microscope, ESEM) Philips Quanta 200. Antes de la toma fotográfica a bajo vacío y sin metalizar, todas las conchas se limpiaron por inmersión en hipoclorito sódico al 5% para eliminar el periostraco y otros residuos orgánicos (microalgas, etc.) y posteriormente con ultrasonidos. Por último, se montaron en un portamuestras para ESEM. Las rádulas se extrajeron por digestión del bulbo bucal en una solución de potasa (KOH) en agua destilada (10g/l) a temperatura ambiente. El mismo proceso se siguió para limpiar los opérculos. A continuación, ambas estructuras se lavaron en agua destilada y se secaron al aire antes de montarlas en un portamuestras y

recubrirlas con una fina capa de oro (10-20 nm) en un metalizador EMITECH K550X, para posteriormente tomar las microfotografías a alto vacío.

Estudios morfométricos

El número de individuos empleados para este estudio está indicado en cada una de las tablas de resultados, variando entre dos y 31 para estudios conquiliológicos y de uno a 14 para la toma de medidas anatómicas. Las variables morfológicas de la concha y del opérculo y las utilizadas para describir la anatomía interna se recogen en la Tabla 6. Para el análisis de las medidas anatómicas y de las conchas se utilizaron los siguientes parámetros estadísticos descriptivos: media, desviación estándar (SD), coeficiente de variación (CV) y valores máximo (Máx) y mínimo (Mín). Todos ellos se obtuvieron empleando el programa SPSS 17.0 para Windows. A la hora de comparar las medidas entre poblaciones, un tamaño muestral diferente podría afectar tanto a la desviación estándar como al coeficiente de variación. Para corregir esta diferencia en ambos parámetros, se han aplicado las correcciones de Holtzman (1950) para la SD y la de Biemann y Kearney (2010) para el CV. Estos cálculos fueron realizados con el paquete MBESS (Kelley y Lai, 2011) para el programa estadístico R (R Development Core Team, 2011).

Con el objetivo de comprobar si las diferencias anatómicas encontradas entre los dos subgéneros (y con la especie *P. (P.) gasulli* por su parte, independientemente) se reflejaban también en las dimensiones de sus estructuras anatómicas o morfológicas, se ha llevado a cabo un análisis discriminatorio (DA) con el programa SPSS 20.0 para Windows. Así, la agrupación inicial de los casos se ha realizado de acuerdo a tres grupos: especímenes de *P. (Corrosella)*, de *P. (Pseudamnicola)* y de *P. (P.) gasulli*. Este tratamiento permite conocer qué variables contribuyen más (las más discriminatorias) a la separación de los grupos, ya que maximiza las diferencias entre los mismos y, al mismo tiempo, minimiza la variabilidad dentro del grupo. Como resultado, esta prueba proporciona por un lado funciones discriminatorias que reúnen la variabilidad encontrada, por otro lado una clasificación jerárquica de las variables en función de su poder discriminatorio, y, por último, un gráfico resumen del análisis.

Tabla 6. Abreviaturas de las variables morfológicas analizadas.

CONCHA		**OPÉRCULO**	
Altura de la abertura	AH	Longitud del núcleo	NL
Longitud de la abertura	AL	Anchura del núcleo	NW
Anchura de la abertura	AW	Longitud del opérculo	OL
Longitud de la vuelta del cuerpo	LBW	Longitud de la última vuelta	OLWL
Número de vueltas de espira	NSW	Anchura de la última vuelta	OLW
Anchura del núcleo de la protoconcha	NPW	Anchura del opérculo	W
			OW
Anchura de la protoconcha	PW		
Longitud de la concha	SL		
Anchura de la concha	SW		
Anchura de la antepenúltima vuelta de espira	WAW		
Anchura de la vuelta del cuerpo	WBW		
Anchura de la penúltima vuelta de espira	WPW		

CTENIDIO, OSFRADIO Y ESTÓMAGO		**SISTEMA REPRODUCTOR MASCULINO**	
Ctenidio	Ct	Próstata	Pr
Osfradio	Os	Pene	P
Saco del estilo	Ss		
Estómago	St		

SISTEMA REPRODUCTOR FEMENINO		**SISTEMA NERVIOSO**	
		Comisura cerebral	CC
Glándula del albumen	Ag	Ganglio cerebral izquierdo	LCG
Bolsa copuladora	Bc	Ganglio pleural izquierdo	LPG
Capsula de la glándula	Cg	Ganglio cerebral derecho	RCG
Conducto de la bolsa copuladora	dBc	Ganglio pleural derecho	RPG
		Conectivo subesofágico	SubC
Oviducto paleal	Po	Ganglio subesofágico	SubG
Receptáculo seminal	SR	Conectivo supraesofágico	SupC
		Ganglio supraesofágico	SupG

Para realizar el análisis discriminatorio, se analizaron nueve variables métricas de la concha (SL/SW, LBW, AH, AL, AW, WBW, WPW, WAW y NSW, abreviaturas en Tabla 6), en un total de 492 ejemplares. Asimismo, y con la misma finalidad, se examinaron las variables anatómicas mostradas en la Tabla 6 y en la Figura 15 en un total de 112 machos y 89 hembras. De esta forma, se pretende verificar si las diferencias encontradas en la morfología de la concha se reflejan

también en la anatomía interna de los ejemplares. Los valores perdidos fueron reemplazados por interpolación lineal. La normalidad de cada una de las variables se evaluó a través de los "tests" de Kolmogorov–Smirnov (para muestras de más de 30 ejemplares) y Shapiro–Wilks (para muestras menores de 30 ejemplares). Para contrastar si el análisis es lo suficientemente robusto a las violaciones de la asunción de la normalidad se aplicó el "F-test" (Lindman, 1974), cuyo valor es dependiente del número de individuos que se esté estudiando. Con el fin de evaluar el poder discriminatorio del análisis se empleó la prueba estadística "λ de Wilk". El valor de este estadístico es igual al cociente de la suma de las varianzas dentro de los grupos y la suma de la varianza total sin distinguir grupos. Este valor refleja el porcentaje de varianza que no se debe a diferencias entre grupos, y oscila entre 0 (las variables tienen alto poder discriminatorio) y 1 (escaso poder discriminatorio).

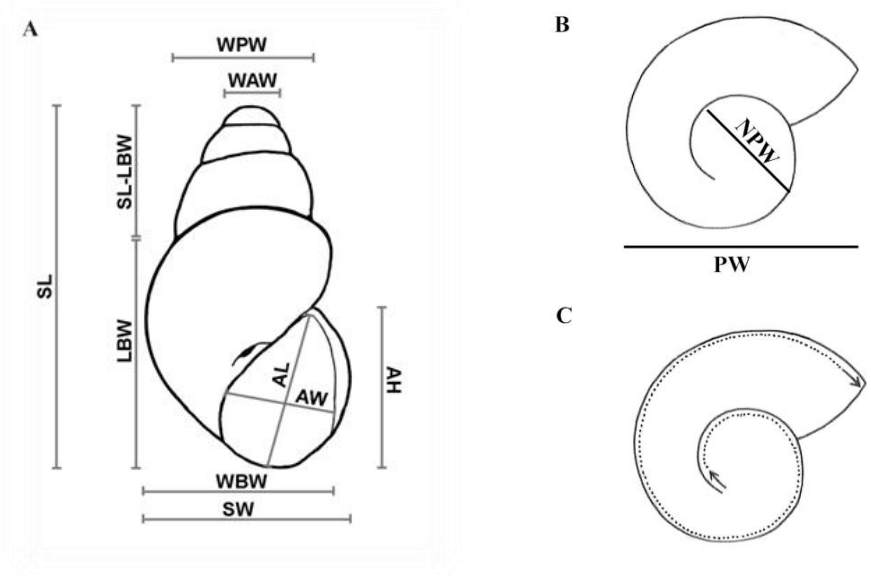


Figura 15. Variables morfométricas examinadas de: **A**. Concha. **B.** Protoconcha. **C.** Método empleado para contar las vueltas de espira de la protoconcha. Variables y método tomados de Ramos *et al*. (2000). Abreviaturas en Tabla 6.

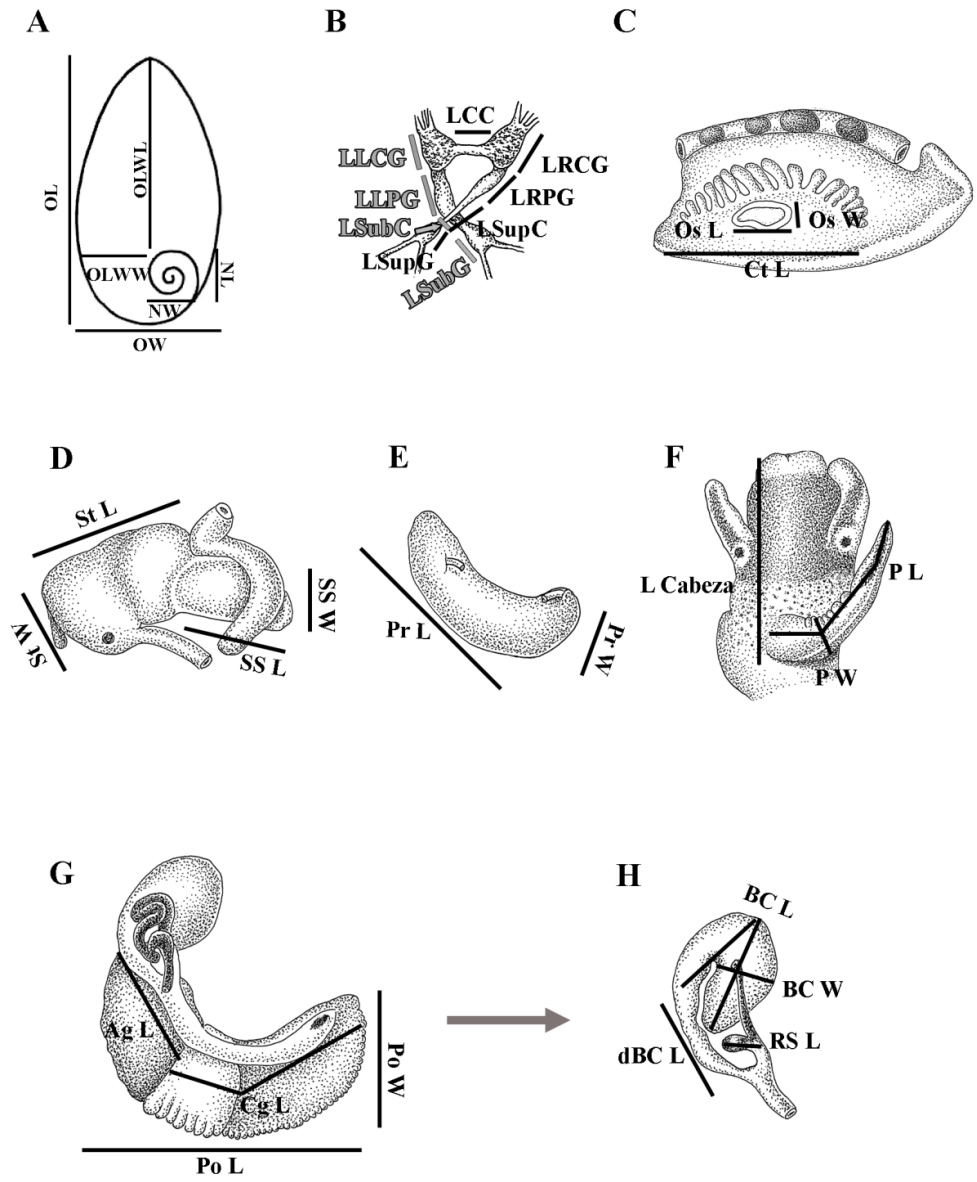


Figura 16. Variables morfométricas analizadas para los caracteres anatómicos. **A.** Opérculo. **B.** Sistema nervioso. **C.** Ctenidio y osfradio. **D.** Estómago. **E.** Próstata. **F.** Cabeza y pene. **G-H.** Sistema genital femenino anterior. Abreviaturas en Tabla 6. Las letras "L" y "W" añadidas a cada variable indican longitud y anchura, respectivamente.

Los estudios moleculares llevados a cabo con ejemplares del género *Pseudamnicola* se han desarrollado en dos fases. La primera comprende el análisis de los individuos de la región íbero-balear que se llevó a cabo en los laboratorios del MNCN. En la segunda fase, y durante una estancia realizada en la Universidad Justus Liebig de Giessen, Alemania, se analizaron y secuenciaron varias poblaciones del género pertenecientes a la cuenca mediterránea. La metodología llevada a cabo en ambas etapas fue muy similar, exceptuando el protocolo de extracción, explicado a continuación. Los dos primeros capítulos de resultados se desarrollaron con el protocolo de extracción llevado a cabo en el MNCN, y el tercero con el que habitualmente se emplea en la Universidad Justus Liebig. Tanto las técnicas moleculares como los análisis filogenéticos fueron los mismos a lo largo de los tres capítulos de resultados; lo único que difiere es el número y tipo de ejemplares (entendido como de uno u otro subgénero). Estas diferencias serán explicadas al inicio de cada capítulo de resultados.

Todos los ejemplares, tratados individualmente, estaban congelados a -80 °C o fijados en etanol de 80%, 96% o absoluto. La técnica molecular para la obtención de las secuencias genéticas comprende tres fases consecutivas: extracción, amplificación y secuenciación de los fragmentos de ADN.

Extracciones de ADN total. Protocolo

Los protocolos de extracción de ADN de las muestras analizadas se seleccionaron para evitar la coprecipitación de mucopolisacáridos junto con el ADN. Esto es debido a la gran cantidad de mucopolisacáridos presentes en sus tejidos que normalmente se aíslan junto con el ADN, encapsulándolo e inhibiendo algunas enzimas tales como la polimerasa, las ligasas y las endonucleasas de restricción 1, 2 y 3 (Sokolov, 2000). Por este motivo, los protocolos de extracción utilizados en el presente trabajo están enfocados a resolver este problema:

- **Protocolo de extracción ChargeSwitch gDNA Micro Tissue** (Invitrogen, Paisley, UK). Se llevó a cabo en el MNCN. Este "kit" es un sistema basado en perlas magnéticas que proporcionan una carga superficial conmutable dependiendo del pH del tampón de dilución, lo que facilita la purificación del ADN. El proceso de extracción completo está representado en la Figura 17. Con el objetivo de preservar el individuo después de la extracción, este protocolo se ha llevado a cabo utilizando una porción del músculo del pie de cada ejemplar. Este tejido proporciona suficiente cantidad de ADN y,

además, evita problemas de contaminación por microorganismos o partículas alimenticias del sistema digestivo.

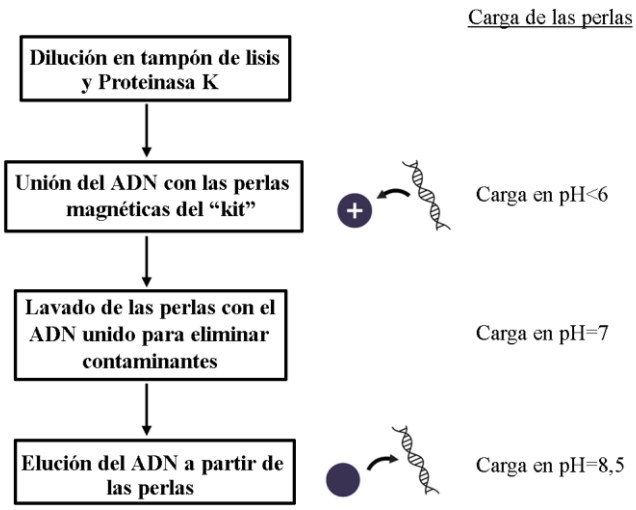


Figura 17. Esquema ilustrativo de cada uno de los pasos aplicados en el protocolo de extracción ChargeSwitch gDNA Micro Tissue.

- **Protocolo específico de gasterópodos, desarrollado por Spolsky *et al.* (1996) y modificado por Wilke *et al.* (2006).** Desarrollado en la Universidad Justus Liebig de Giessen. En este caso se empleó el animal completo, rompiendo la concha y utilizando las partes blandas. Cada uno de los individuos conservados en etanol de 80° se sumergió durante 10 minutos en un tampón de lavado (0,02 M tris (hidroximetil) aminometano (TRIS) base, 0,1 M ácido etilendiaminotetraacético (EDTA), pH 8.0) frío. A continuación, se cortaron las partes blandas del individuo en pequeños trozos y se incubaron toda la noche en un baño de agua a 58 °C en 200 µl de tampón de lisis (0,02 M TRIS base, 0,1 M EDTA, 0,5% Sarkosyl, pH 8,0) y 3 µl de 20 µg/µl de proteinasa K. Después de la digestión, se añadieron 35 µl de NaCl 5 M y 35 µl de una solución de 5% de bromuro de cetiltrimetilamonio (CTAB)/0,5 M NaCl. La extracción se llevó a cabo con 270 µl de cloroformo. Después de la centrifugación durante 5 min a 9.000 rpm, se transfirió el sobrenadante a un tubo nuevo, al que se añadieron además 270 µl de tampón de precipitación CTAB (1% CTAB, 0,05 M TRIS base, 0,01 M EDTA), se mezclaron y la mezcla se dejó en reposo durante 45 min a temperatura ambiente. Pasado ese tiempo, la mezcla se volvió a centrifugar, esta vez 10 min a 12.000 rpm, desechando el sobrenadante y

redisolviendo el precipitado en 100 µl de NaCl/TE (0,01 TRIS base, 0,001 M EDTA, 1 M NaCl, pH 8,0) y 1 µl de 10 mg/ml RNasa. Después de la incubación durante 8 min a 65 °C, se añadieron 250 µl de etanol 96° frío y se dejó reposar toda la noche para que precipitara el ADN. Al día siguiente, se centrifugó durante 15 min a 12.000 rpm y después se limpió ese precipitado dos veces con 300 µl de etanol frío de 70°, dejándolo secar a temperatura ambiente durante 5-10 min. Finalmente, se resuspendió en aproximadamente 50 µl de agua ultrapura.

Amplificación de los genes a estudiar

La amplificación de los genes objeto de este estudio se realizó a través de la técnica de la reacción en cadena de la polimerasa, conocida como PCR por sus siglas en inglés (Polymerase Chain Reaction). Con esta técnica se consigue obtener un alto número de copias de una región específica del ADN y así, tras su amplificación, se obtiene la cantidad necesaria para su secuenciación. Su fundamento radica en la propiedad natural de la enzima polimerasa del ADN, que es capaz de replicar hebras de ADN empleando ciclos donde se alternan tres temperaturas distintas: la de desnaturalización de la doble hebra de ADN (sobre los 94-95 °C), la de ligamiento del cebador con una de las cadenas de ADN (normalmente entre 40 y 60 °C) y la de elongación (habitualmente, 72 °C).

El avance y la generalización de esta técnica se debió a la utilización de ADN polimerasas termoestables. Así, la fase de desnaturalización, que se produce a elevadas temperaturas, generalmente a 94 °C, no conlleva riesgo de degradación de la enzima. La condición de termoestabilidad se consiguió empleando polimerasas extraídas de microorganismos adaptados a vivir a altas temperaturas (Mullis, 1999). Así, en esta tesis la polimerasa utilizada ha sido la Taq polimerasa, extraída de la bacteria termófila *Thermus aquaticus*.

Elección de los cebadores. En la fase de ligamiento la temperatura desciende para permitir la unión del cebador a la hebra de ADN. Esta temperatura es específica del marcador molecular que se utilice. Un cálculo aproximado de esta temperatura se realiza a partir del número de pares G-C y A-T del cebador, ya que la primera combinación está unida por tres puentes de hidrógeno y requiere mayor temperatura para formarse y separarse. Los genes elegidos para llevar a cabo el estudio filogenético de este grupo han sido:

a) Los genes mitocondriales citocromo c oxidasa subunidad I (COI) y el gen codificante para la subunidad grande del ARN ribosómico (ARNr 16S).

b) Los genes nucleares que codifican para la subunidad grande y pequeña del ARN ribosómico (ARNr 28S y ARNr 18S, respectivamente).

Una de las principales razones por la que se han elegido estos marcadores genéticos ha sido porque en estudios previos sobre filogenias de hidróbidos estos genes marcadores han resultado ser resolutivos a nivel intragenérico e intergenérico (Wilke *et al.*, 2000a; Wilke *et al*., 2001; Liu y Hershler, 2005). Por otro lado, la disponibilidad de secuencias de estos genes de *Pseudamnicola* en bases de datos como "Genbank" hace posible realizar estudios comparativos y añadir así mayor número de taxones al análisis.

Una vez elegidos los genes, se buscaron los cebadores para obtener las secuencias de esos fragmentos. La relación de cebadores, la secuencia nucleotídica de cada uno de ellos y la fuente de donde se han extraído se indica en la Tabla 7.

Tabla 7. Relación de los cebadores estudiados con su secuencia nucleotídica y la fuente donde se ha encontrado esa secuencia.

Cebadores	Dirección	Secuencia del cebador	Referencia
16Sar-L	Forward	CGACTGTTTAWCAAAAACAT	Palumbi *et al.* (1991)
16Sbr-H	Reverse	CCGGTCTGAACTCAGATCA	Palumbi *et al.* (1991)
LCO1490	Forward	GGTCAACAAATCATAAGATATTGG	Folmer *et al*. (1994)
COI-H	Reverse	TCAGGGTGACCAAAAAAYCA	Davis *et al.* (1998)
28S F63	Forward	ACCCGCTGAAYTTAAGCATA	Park y Ó Foighil, 2000. Modificado por Benke *et al*., 2009
28S LSU3	Reverse	TCCTGAGGGAAACTTCGG	Park y Ó Foighil (2000) modificado
18S	Forward	GCCAGTAGCATATGCTTGTCTC	Holland *et al.* (1991)
18S	Reverse	AGACTTGCCTCCAATGGATCC	Holland *et al.* (1991)

Protocolo de preparación de reactivos. Cada mezcla de reacción de la PCR, para un volumen total de 25 µl, contenía 0,5-3 µl de ADN, 2,5 µl del correspondiente tampón (con 10 × 2 mM $MgCl_2$), 0,5 µl de dNTPs (10 mM), 0,5 µl de cada cebador (10 µM), 0,25 µl de $MgCl_2$ (50 mM), 0,5 µl de Taq ADN polimerasa (5U/µl) (Biotools) y agua bidestilada. Las condiciones para la PCR con los distintos genes están recogidas en la Tabla 8. Cabe destacar que se han añadido 5 pre-ciclos a una temperatura de hibridación menor (42 °C) para facilitar el ligamiento del cebador con el ADN, ya que en ocasiones esta unión se ve interferida por la presencia de polisacáridos.

Procesamiento de los productos de PCR. Posteriormente se tomaron 5 µl del volumen total de cada muestra amplificada para determinar la presencia y cantidad de ADN amplificado en un gel de agarosa al 1%, teñido con SYBR Safe (Invitrogen), para poder visualizar el ADN al exponer el gel bajo luz blanca con filtro azul.

Tabla 8. Secuencias de tiempos y temperaturas para los cuatro marcadores estudiados.

ARNr 16S	**COI**	**ARNr 28S**	**ARNr 18S**
94 °C (4 min)	94 °C (4 min)	94 °C (4 min)	94 °C (4 min)
5 ciclos:	5 ciclos:	5 ciclos:	5 ciclos:
94 °C (1 min)	94 °C (1 min)	94 °C (1 min)	94 °C (1 min)
42 °C (1 min)	42 °C (1 min)	42 °C (1 min)	42 °C (1 min)
72 °C (1 min)	72 °C (1 min)	72 °C (1,5 min)	72 °C (1 min)
35 ciclos:	35 ciclos:	35 ciclos:	35 ciclos:
94 °C (45 s)	94 °C (45 s)	94 °C (40 s)	94 °C (45 s)
50 °C (45 s)	48 °C (45 s)	51 °C (40 s)	46 °C (45 s)
72 °C (45 s)	72 °C (45 s)	72 °C (1,5 min)	72 °C (45 s)
72 °C (10 min)	72 °C (10 min)	72 °C (10 min)	72 °C (10 min)

Secuenciación

Los fragmentos amplificados se purificaron mediante precipitación con etanol y fueron secuenciados por el método "BigDye Terminator" (Applied Biosystems, ABI) en una empresa de secuenciación (SECUGEN S.L.).

Métodos utilizados en la reconstrucción filogenética

Una vez obtenidas las secuencias para cada individuo, todas ellas fueron editadas para los análisis posteriores con los programas Sequencher v. 4.6 (Gene Code Corporation) y Bioedit v. 7.0.5.3 (Hall, 1999), con los que además se eliminó la región de los cebadores. Aquellos genes no codificantes de proteínas, es decir ARNr 16S y ARNr 28S, fueron alineados con los programas PAUP* 4.0a123 (Swofford, 2002) y Se-Al versión 2.0a11 (Rambaut, 2002). Durante esta fase no se encontraron regiones demasiado ambiguas, por lo que el alineamiento se pudo realizar manualmente.

Los cuatro genes estudiados en el presente trabajo se analizaron por separado, pero también conjuntamente. Para estudiar el nivel de congruencia de los datos al combinar diferentes genes, las matrices combinadas se analizaron a través de la aplicación "Incongruence length difference test" (ILD) del programa PAUP (Mickevich y Farris, 1981; Farris *et al.*, 1994). Aunque este "test" es normalmente utilizado como punto de partida para comparar diferentes matrices de datos, algunos autores discrepan en su validez como único criterio de congruencia de los datos (Barker y Lutzoni, 2002; Hipp *et al.*, 2004). La razón de este hecho parece ser que el "test" IDL es susceptible de cometer un error de tipo I, es decir, cuando la hipótesis nula, la cual asegura que existe concordancia entre las genealogías, se rechaza siendo verdadera. Este hecho podría darse, ya que este "test" está basado en la parsimonia y, cuando las diferentes particiones presentan disparidades en los niveles de homoplasias y/o diferentes tasas evolutivas, puede resultar significativo (Barker y Lutzoni, 2002; Hipp *et al.*, 2004) y mostrar que los datos no son congruentes. Sin embargo, esto no significa que las topologías de los distintos genes no sean compatibles y que la combinación de las mismas no proporcione una filogenia robusta.

La información filogenética contenida en las matrices de secuencias ha sido estudiada con diferentes tratamientos, los cuales están basados en diferentes hipótesis acerca de los procesos evolutivos. En algunos de ellos, como en la parsimonia, tales suposiciones se encuentran implícitas, y en otros, como en la máxima verosimilitud y los análisis bayesianos, son explícitas. Como estas últimas inferencias se basan en probabilidades, la obtención de un modelo evolutivo que se ajuste a los datos es esencial para estimar las relaciones filogenéticas entre taxones.

Así, a través del programa JModeltest versión 0.1.1 (Posada, 2008) bajo el criterio de información de Akaike (AIC; Akaike, 1974), se procedió a evaluar el modelo evolutivo que mejor se ajustase a los datos. El programa JModeltest permite comparar diferentes hipótesis o modelos de sustitución de ADN por medio del estudio jerárquico de las distintas hipótesis nulas y alternativas que se prueban en cada caso, lo que confiere una descripción estadística al proceso estocástico. El nivel de significación es de 0,01 y los modelos que van a ser comparados, evaluando su verosimilitud (probabilidad), consisten en distintas combinaciones, desde las más simples a las más complejas, de únicamente cuatro parámetros: frecuencia de las bases, tasas de cambio (relaciones entre transiciones y transversiones), tener o no en cuenta la proporción de los sitios invariables y, por último, la tasa de variación entre sitios (la homogeneidad en el reparto de los cambios a lo largo de la secuencia).

Las inferencias filogenéticas mostradas en este trabajo han sido obtenidas por diferentes métodos, cuyo fundamento y metodología son explicados a continuación:

- **Métodos basados en distancias genéticas**: Las secuencias cambian a lo largo del tiempo, acumulando sustituciones con respecto a las secuencias "originales" y, por lo tanto, cuando dos de ellas derivan de un ancestro común y evolucionan independientemente una de otra, se dice que han divergido. La medida de esa divergencia es lo que se llama **distancia genética** (Strimmer y von Haeseler, 2009). El método basado en distancias genéticas llevado a cabo en este trabajo es el de "Neighbour-joining" (NJ) (Saitou y Ney, 1987). Este método produce árboles no enraizados y no asume una tasa de evolución constante entre linajes. Se calcula la longitud total del árbol, resultante de la suma de la longitud de cada una de las ramas para cada topología y se toma como árbol definitivo aquél que presente menor longitud total. Como se ha indicado, en estos métodos se parte de índices (distancias o divergencias) que indican la diferencia entre las secuencias analizadas. Todas las sustituciones, en cada posición, serán reflejadas en ese índice medio, perdiéndose la información de cada uno de los "caracteres". De esta forma, los métodos basados en distancias se convierten en métodos "fenéticos", en los que se comparan similitudes. Por tanto, sus resultados no pueden considerarse realmente como propuestas o inferencias filogenéticas, aunque son de utilidad por varios motivos, como la rapidez en su cálculo y la posibilidad de establecer ciertos criterios de diferenciación medios de cara a la comparación con diversos estudios o taxones. La longitud de las ramas se calcula con el algoritmo de Fitch y Margoliash (Fitch y Margoliash, 1967).

- **Métodos basados en caracteres discretos:** En estos métodos, las relaciones entre las UTOs (Unidades Taxonómicas Operacionales) están determinadas

por cada uno de los caracteres que las definen. Para datos moleculares, los caracteres en la matriz son cada una de las posiciones de la secuencia, y los estados de carácter son las cuatro bases nucleotídicas, pudiéndose considerar como quinto estado de carácter a los fenómenos de inserciones y deleciones. Los métodos basados en caracteres utilizados en el presente trabajo fueron:

- **Máxima parsimonia (MP):** Basado en el principio de búsqueda de la topología o colección de topologías, que minimicen el número de cambios evolutivos, entendiéndose éstos como cambios de un estado de carácter a otro. Así, esos cambios se describen como diferencias observadas en cada posición informativa de las UTOs (Kolekar *et al.*, 2010). De esta forma, se considera más adecuada la topología que presente un mayor "ahorro evolutivo" en cuanto al número de cambios. Éstos son calculados para cada topología a través del algoritmo de Fitch (Fitch, 1971). Este método buscará, pues, la topología en la que la suma de "pasos" (cambios de los caracteres) sea menor, minimizando por tanto el número de cambios acontecidos para explicar las diferencias entre los taxones. Aquellas posiciones (caracteres) de un gen que no presentan variaciones en todas las UTOs estudiadas, se denominan caracteres no informativos y aquéllas que cambian sólo en un taxón (autapomorfías) son consideradas "parsimoniosamente no informativas". Los estados de carácter que sean compartidos por más de un taxón serán considerados como caracteres derivados compartidos y éstos serán los que determinen la topología del árbol. Este análisis se realizó con el programa PAUP, a través de un análisis heurístico, en el que se aplicó el denominado intercambio de ramas ("Branch swapping") y eligiendo la opción TBR ("Tree Bisection Reconection"), para mejorar la búsqueda, ante la imposibilidad de analizar todos los árboles posibles. Con esta modalidad, se van añadiendo progresivamente taxones, se divide el árbol en dos subárboles por un nodo interior y se intentan todas las conexiones posibles entre las ramas de los dos árboles creados, eligiendo el de menos longitud y así sucesivamente, hasta incluir todos los taxones estudiados.

  Para desarrollar todos los pasos de esta inferencia filogenética, así como de las otras dos explicadas a continuación, es necesario establecer la polarización de los caracteres y eso se consigue enraizando el árbol filogenético. Entre los diferentes métodos de enraizamiento existentes, en la presente tesis la inclusión de grupos externos se ha empleado para tal fin. La elección de los mismos, parte crucial en el análisis, se ha llevado a cabo en base a dos criterios: incluir un taxón del grupo hermano más próximo, en este caso la especie *Peringia ulvae*

(Pennant, 1777), perteneciente a la subfamilia Hydrobiinae (grupo hermano de Pseudamnicoliinae) y un taxón de un grupo filogenéticamente más lejano, como la especie de hidróbido *Mercuria emiliana* (Paladilhe, 1869).

- **Máxima verosimilitud (ML):** Método desarrollado por Felsenstein (1981). Está basado en un enfoque probabilístico, ya que elige aquella topología que presenta una mayor probabilidad de explicar la información aportada por los datos. Así, se asigna para los datos un modelo y una hipótesis. La posible hipótesis incluye una serie de estructuras topológicas, longitud de ramas y parámetros que se infieren del modelo evolutivo de las secuencias. Asignando valores a esos elementos es posible computar la probabilidad de los datos (Schmidt y von Haeseler, 2009) y así comparar cuál es la topología más probable. Este método no asume un reloj molecular. Fue llevado a cabo con el programa PHYML v2.4.4 (Guindon y Gascuel, 2003), empleando el modelo seleccionado por jModeltest.

La técnica utilizada para evaluar la robustez de las hipótesis filogenéticas inferidas en estos dos análisis ha sido el análisis no paramétrico "Bootstrap" (Felsenstein, 1985). En este método de re-muestreo se crean nuevas matrices a partir de la matriz original tomando al azar columnas (caracteres) de ésta hasta alcanzar el mismo número de caracteres que la matriz original. El re-muestreo se realiza con reposición, lo que quiere decir que en cada nueva matriz hay caracteres que pueden no aparecer, mientras que otros estarán duplicados, de forma que se evalúa, en cierto sentido, la congruencia de la señal filogenética de los distintos caracteres. En esta tesis la técnica "bootstrap" se ha llevado a cabo mediante la alternativa heurística (1.000 réplicas) y el resultado final se resume y visualiza en un único árbol obtenido por la técnica "Majority-rule consensus tree".

- **Métodos basados en entorno bayesiano:** Basado en el teorema de Bayes, en el cual se emplean las observaciones o probabilidades iniciales para inferir la probabilidad final de que una hipótesis pueda ser cierta. Se considera que el método bayesiano representa el auténtico método científico, ya que, aunque evalúa también la verosimilitud de los árboles como en el método anterior, parte de los datos y evalúa la propuesta filogenética (árbol), es decir, la adecuación de ésta a los datos iniciales, y no al revés, como se da en la máxima verosimilitud, siempre en ambos casos teniendo en cuenta un modelo. La otra gran diferencia con respecto a dicho método es que aquí se tienen en cuenta todos los valores posibles de los parámetros que entran en juego, mientras que en el anterior se selecciona un único valor. De esta

forma, el número de valores de parámetros es tan ingente, que se requiere una aproximación para poder llevar a cabo los cálculos. Así, la inferencia bayesiana (BI) utiliza la combinación de las cadenas de Markov y del método de Monte Carlo (MCMC) (Metropolis *et al.*, 1953; Hastings, 1970), para permitir dichos cálculos en combinación con el modelo elegido y los datos para generar una distribución de probabilidad a posteriori de los árboles. Esta distribución a posteriori describe la probabilidad de cada árbol teniendo en cuenta la distribución a priori de los datos y el modelo seleccionado. Esta distribución de los árboles es el principal producto de los análisis filogenéticos bayesianos (Archibald *et al*., 2003). Para obtener la distribución de probabilidad posterior se ha utilizado el programa MRBAYES 3.1.2 (Huelsenbeck, 2000; Huelsenbeck y Ronquist 2001), con el cual se generaron 5 millones de réplicas (árboles) en dos carreras paralelas, tomando un árbol cada 1.000 generados. En este muestreo de árboles filogenéticos, aquellos creados durante los primeros pasos del análisis suelen ser los que poseen menores probabilidades posteriores (BPP), así que son descartados (generalmente se descarta el 10%) y se toma el resto para hacer un árbol de consenso que sintetizará todos los árboles muestreados.

Estimación de los tiempos de divergencia

Una vez inferidas las relaciones filogenéticas de las especies estudiadas de *Pseudamnicola*, se procedió a estimar los tiempos de divergencia de las mismas a través de la técnica del reloj molecular. Esta herramienta es utilizada para relacionar el número de mutaciones fijas (= sustituciones) en las secuencias de nucleótidos o aminoácidos con el tiempo de divergencia de los taxones (Wilke *et al*., 2009). La introducción del concepto de reloj molecular es atribuida a Zuckerkandl y Pauling (1962), que encontraron diferencias en los aminoácidos de las cadenas α y β de la hemoglobina de los mamíferos y comprobaron que esos cambios eran aproximadamente proporcionales a los tiempos de divergencia inferidos a partir de datos paleontológicos. Los métodos de datación genética, es decir, la estimación de los tiempos de divergencia de dos linajes procedentes de un ancestro común sobre la base de secuencias de nucleótidos o aminoácidos, pueden ser aplicados tanto en estudios de genética de poblaciones como en filogenias. En los estudios de genética de poblaciones, el marco coalescente se utiliza para calcular la "edad" del ancestro común más reciente (MRCA) de un número de alelos. Esa edad del MRCA se mide en número de generaciones. Esta aproximación retrocede en el tiempo y se basa en la suposición de que un par de alelos se unen, es decir, encuentran su MRCA, en algún punto en el tiempo en el pasado (Edwards y Beerli, 2000). En genética de

poblaciones, estos principios se aplican para la estimación de tiempos de divergencia dentro de una especie.

Para la estimación de tiempos de divergencia entre especies o grupos de especies se han sugerido varias metodologías. Mientras que en los primeros estudios aplicando un reloj molecular las matrices de distancia genética fueron utilizadas para estimar las tasas de sustitución, hoy en día estas tasas suelen ser derivadas de la filogenia, o de la secuencia de datos en relación con las topologías de los árboles (Rutschmann, 2006). Esta última aproximación utiliza la topología de las ramas junto con la información de la longitud de las mismas para estimar la profundidad de nodo (d_N, número de sustituciones por sitio). Junto con la tasa de sustitución (λ en número de sustituciones por posición), el tiempo de divergencia (t_D) se calcula como: $t_D = d_N / \lambda$. Algunos especialistas, sin embargo, utilizan para la estimación de tiempos de divergencia las distancias genéticas no en términos de profundidad de nodo, sino en términos de longitud total de las ramas entre dos taxones. Como esa longitud total es igual a la profundidad del nodo × 2, las tasas de sustitución tienen que ser modificadas en consecuencia y, en estos casos, por lo general las tasas de divergencia (tasa de divergencia= tasa de sustitución × 2) se usan como "velocidades" de reloj molecular.

A fin de calcular los tiempos de divergencia de las profundidades de nodos, la tasa de sustitución tiene que ser estimada a partir de las fechas de origen externo. Esto es más o menos factible cuando se trabaja con poblaciones actuales (como en los casos de los estudios epidemiológicos), pero para el análisis del tiempo de divergencia entre especies se requiere una aproximación indirecta. Esto se puede hacer con la información de los fósiles o de eventos biogeográficos (Bromham y Penny, 2003). Además, el uso de múltiples puntos de calibración es más efectivo para obtener estimas y tiempos de divergencia más creíbles (Donoghue y Benton, 2007; Bidegaray-Batista y Arnedo, 2011). A través de estudios del registro fósil, se ha podido averiguar que la famila Hydrobiidae tuvo su origen en el periodo Carbonífero en Laurasia (Knight *et al*., 1960; Kabat y Hershler, 1993; Arconada y Ramos, 2003). Sin embargo, Ponder (1988) cuestionó la clasificación de esos fósiles como hidróbidos. Debido a la incertidumbre de este punto de calibración, las edades de diversificación en este trabajo fueron estimadas a partir de varios puntos biogeográficos de calibración ya publicados para hidróbidos. Así, la tasa específica aplicada para el gen COI es de 0,81% ± 0,24% sustituciones por linaje por millón de años. Esta tasa media integra aquellas tasas publicadas para hidróbidos en Wilke (2003); Hershler y Liu, (2008) y Wilke *et al*. (2009). Asimismo, aunque diferentes loci posean diferentes genealogías, son transmitidos en el mismo proceso y se espera que compartan algunas de sus trazas. Por lo tanto, se han incluido en el mismo análisis los fragmentos de los otros dos genes estudiados (16S y 28S) para estimar su

tasa de sustitución y a la vez reforzar la reconstrucción de los tiempos de divergencia.

Para poder obtener el árbol ultramétrico y las estimaciones de los tiempos de divergencia se ha utilizado el programa BEAST ver. 1.7.1 (Drummond *et al.*, 2012) implementado con *BEAST (Helend y Drummond, 2010). Este paquete de programas trabaja bajo entornos bayesianos. El fichero de entrada para este programa fue preparado con la interfaz BEAUti ver. 1.7.1. Se utilizaron las matrices para cada gen (COI, 16S y 28S) con las secuencias de *Pseudamnicola (Pseudamnicola)* (Sección primera de Resultados), *Pseudamnicola (Corrosella)* (Sección segunda de Resultados), y del género completo (Sección tercera de Resultados), cuyas características (esto es longitud, número de individuos, etc.) están detalladas en cada una de estas secciones de resultados. Los pasos para realizar el análisis han sido:

- Primero se aplicó un reloj molecular estricto y luego uno relajado y se compararon los resultados. Esto se llevó a cabo con la matriz del COI (ya que los puntos de calibración son para este gen) y con el resto de matrices (añadiendo el 16S y 28S), para estimar además las tasas de mutación de estos dos genes.

- Para comprobar si las tasas de mutación han permanecido constantes a lo largo del tiempo (reloj molecular estricto) o no (reloj relajado), se ha aplicado un "test" relativo de diversificación (RRT, Relative Ratio Test) (Takezaki *et al.*, 1995) sobre todas las ramas para detectar posibles aceleraciones en algunos puntos del árbol. Este análisis se ha realizado con el programa PHYLTEST 2.0 (Kumar, 1996). Después de detectar variaciones en las tasas evolutivas, el análisis se ha llevado a cabo siguiendo un reloj molecular relajado.

- Para el reloj molecular relajado se ha seguido el tipo "uncorrelated log-normal" (Drummond *et al.*, 2006), donde las tasas de mutación de las ramas no están relacionadas, es decir, evolucionan independientemente.

- Además de las especiaciones, se han tenido en cuenta las extinciones (aplicación del modelo "birth-death" de Gernhard, 2008).

- Los modelos de sustitución nucleotídica para cada gen fueron tomados de los resultados del jModelTest.

- Se realizaron un total de 50 millones de generaciones de las cuales se tomó un árbol de cada 2.000 y se descartó el 10% inicial de esta muestra, momento en el que se alcanzó la estabilización de los valores de los parámetros.

- Con el programa Tracer ver. 1.5 (Rambaut y Drummond, 2009) se estudiaron los resultados obtenidos del programa BEAST, ya que proporciona los parámetros que caracterizan a los árboles resultantes y el tamaño efectivo de muestra (ESS, Effective Sample Size) que poseen dichos parámetros. Para que un parámetro se considere válido es necesario que el valor de ESS sea mayor de 200.

- Al igual que con los análisis bayesianos, se hicieron dos carreras paralelas de 50 millones de generaciones y se combinaron tanto los parámetros como los árboles gracias al programa LogCombiner ver. 1.7.1.

El árbol consenso de todos los árboles muestreados fue obtenido con el programa TreeAnnotator ver. 1.7.1 y este árbol pudo ser visualizado en FigTree ver. 1.3.1 (Rambaut, 2009). Este programa permite además representar las edades estimadas de los nodos.

Estudio de posibles factores de diversificación

Con el fin de comprobar la influencia que podrían tener algunas variables ambientales y geográficas sobre la divergencia genética de las especies, se realizó independientemente un "test" de Mantel (Mantel, 1967) a través del paquete Vegan (Oksanen *et al*., 2012) del programa estadístico R (R Development Core Team 2011). Con este "test" se estudió la correlación, en tres diferentes ensayos, entre la matriz de distancia genética entre poblaciones (medidas como distancias no corregidas) y las matrices de diferencias en conductividad o en altitud y de distancia geográfica. El nivel de significación fue testado basado en 9.999 permutaciones. Debido al escaso caudal que caracteriza a las localidades donde habita el género *Pseudamnicola* (principalmente fuentes y arroyos), otro parámetro frecuentemente analizado, la temperatura del agua de la localidad, no ha sido considerado para este análisis, ya que la temperatura podría estar influenciada por las variaciones estacionales de la temperatura del aire. Además, como las poblaciones fueron recolectadas en diferentes periodos del año, no sería consistente comparar dichas medidas.

RESULTADOS

1. TAXONOMÍA Y FILOGENIA DEL SUBGÉNERO *PSEUDAMNICOLA (PSEUDAMNICOLA)* EN LA REGIÓN ÍBERO-BALEAR

GENUS *PSEUDAMNICOLA* PAULUCCI, 1878

Type species

Bithynia lucensis Issel, 1866 (Kennard and Woodward, 1926), subsequent designation.

Etymology

The name *Pseudamnicola* was used to distinguish the European species formerly included in *Amnicola* (Paulucci, 1878).

Diagnosis (*sensu* Delicado, Machordom and Ramos, 2012)

Shell ovate-conic, with an inflated body whorl about three-quarters of shell length and aperture complete and oval; operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral with submarginal nucleus; ctenidium well-developed; osphradium elliptical of intermediate width; radula with central tooth having one basal cusp on each lateral margin and a basal tongue broadly V-shaped; stomach with two chambers and a thin caecum lying on ventral side of posterior chamber; one digestive gland opening in posterior chamber; renal oviduct pigmented; only one seminal receptacle arising from renal oviduct close to insertion of bursa copulatrix duct; bursa copulatrix well-developed protruding behind pallial oviduct; prostate gland bean-shaped and penis simple with a distal patch of pigmentation, variable in size; nervous system pigmented with supraoesophageal connective longer than suboesophageal.

Composition

According to Boeters (1984), the genus is composed of two subgenera: *Pseudamnicola s. str.* and *Corrosella* (Boeters, 1970).

SUBGENUS *PSEUDAMNICOLA (PSEUDAMNICOLA)* PAULUCCI, 1878

Type species

Bithynia lucensis Issel, 1866 (Kennard and Woodward, 1926), subsequent designation

Diagnosis

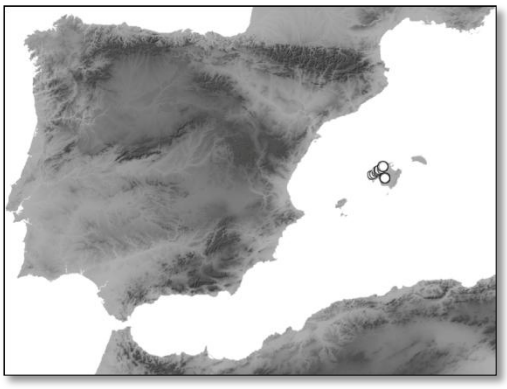
Shell ovate-conic, slightly longer than wide and with an aperture wider than long; female genitalia with pyriform bursa copulatrix and one elongate seminal receptacle; penis broadly triangular with the base expanded and many folds along its entire surface; penis with a dark patch of pigment, whose extension varies among species, from its middle region to the tip; nervous system generally elongate (RPG ratio from 0.50 to 0.67).

Composition

Six species in the Ibero-Balearic region: two in the Iberian Peninsula (one of which is also present in Ibiza Island), three in Majorca Island, and one in Minorca Island.

Pseudamnicola (Pseudamnicola) beckmanni Glöer and Zettler, 2007

Pseudamnicola tramuntanae
Altaba, 2007: 27.



Type locality

Fountain in Deià, former washhouse on the outskirts of Deià, Majorca, 39.746º N, 2.649º E (Glöer and Zettler, 2007).

Type material

Holotype (ZMH 51012) and five paratypes (ZMH 21013) in Zoologischen Museum, Hamburg; nine paratypes in K.-H. Beckmann's collection, five in Glöer's collection and 60 in Zettler's collection.

Localities

After finding the washhouse in Deià (type locality) completely dry in 2008, the specimens studied here were collected at El Rentador Spring (type locality of the junior synonym *P. tramuntanae*), near the washhouse on the outskirts of Deià. The species has also been found in other localities on Majorca Island (see Appendix II).

Material examined for morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Tables 1-7) taken from specimens from El Rentador Spring, Majorca. Males and females specimens were collected in April 2008.

New diagnosis

Shell with yellowish periostracum, last body whorl occupying around four-fifths of shell length and aperture slightly longer than wide; protoconch microsculpture with small pits; central tooth of radula with five wide, lateral cusps at each side; black pigmentation on intestine; pyriform bursa copulatrix; elongate seminal receptacle; renal oviduct black pigmented until the base of seminal receptacle; triangular penis with many folds over the entire surface and a small patch of pigmentation on its distal region; nervous system brown pigmented, ganglia darker than connectives; supraoesophageal connective at least eight times longer than suboesophageal; RPG ratio around 0.55.

Description

Shell ovate-conic (Figures 18A, D, G), yellowish with 3.5-4.25 spire whorls and around 2-3 mm in height (Appendix III: Table 1); protoconch approximately 375 µm in width with 1.5 whorls and a nucleus around 150 µm in length (Figure 19A); protoconch with small pits and some folds near apex (Figure 19B); last body whorl about four-fifths of total length; convex whorls and deep sutures; inner lip wider than outer; the edge of peristome is simple and straight (Figure 18D).

Operculum with approximately 2.5 spire whorls (Figures 20A, B; Appendix III:

Table 2) and a muscle attachment area on the internal side oval located near the nucleus.

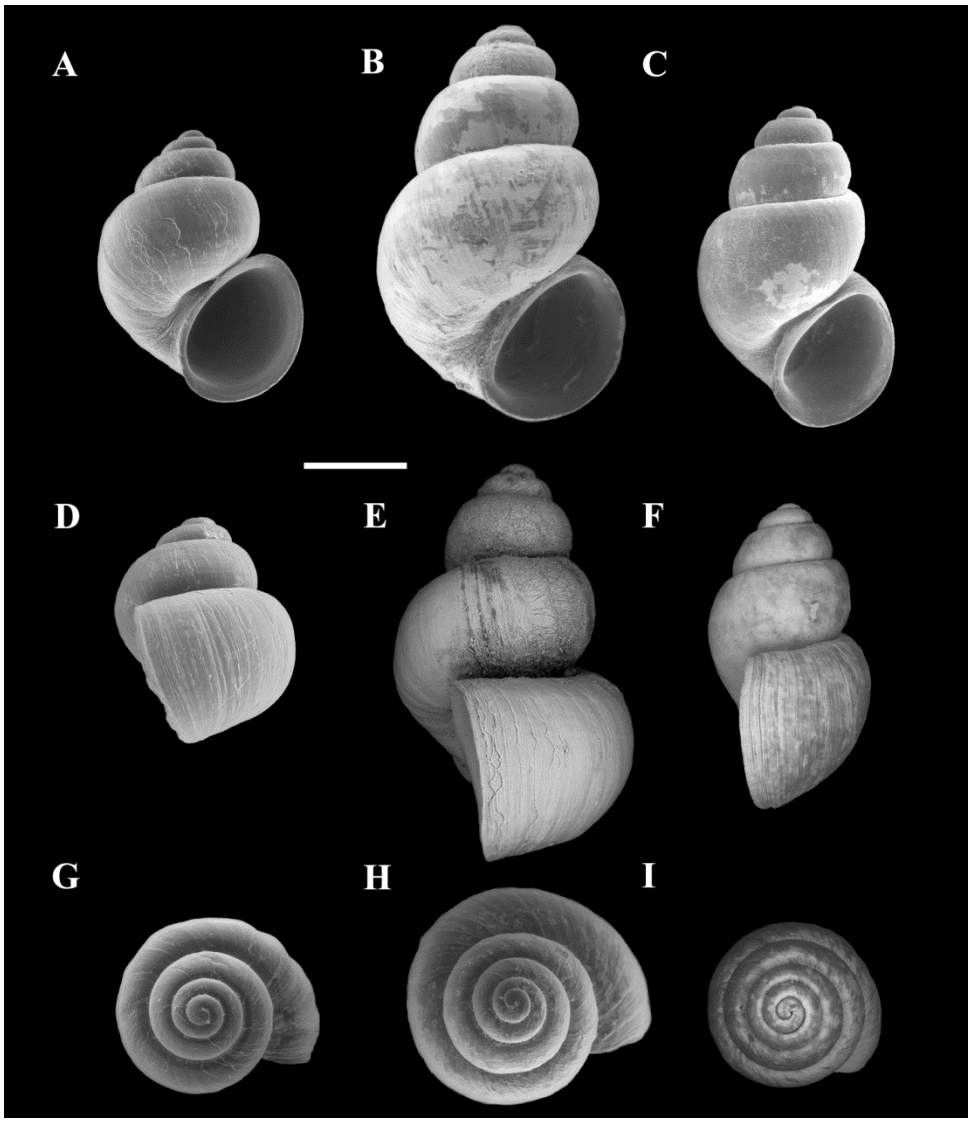


Figure 18. Front, lateral and apical views of shells of Majorcan species. **A, D, G.** Shells of *Pseudamnicola (P.) beckmanni* from El Rentador Spring, Deià. **B, E, H.** Shells of *P. (P.) granjaensis* from La Granja, Esporles. **C, F, I.** Shells of *P. (P.) artanensis* collected from a ditch near Betlem Hermitage, Artá.

Radula size medium (24%) relative to maximum shell dimension and six times longer than wide (Figure 21A, Appendix III: Table 3); around 55 rows of teeth; central tooth with a wide median cusp and five small lateral cusps (Figure 21D,G);

basal tongue V-shaped; lateral teeth with three relatively tapered lateral cusps; inner marginal tooth contains approximately 15 cusps of decreasing size; outer marginal tooth with around 20 cusps smaller than inner marginal cusps (Figure 21J).

Pigmentation and anatomy. Head with uniform brown pigment from snout to base of penis; pigmentation lighter on neck; ocular region lacks pigmentation (Figure 22D); snout as long as wide with medium distal lobation; foot size intermediate with dorsal pigmentation. Ctenidium with 19-22 well-developed gill filaments situated in the middle of the pallial cavity; osphradium around 30% of ctenidium length, located in the opposite middle of the ctenidium (Figure 23A, Appendix III: Table 4). Stomach slightly wider than long with a medium-sized gastric caecum (Appendix III: Table 4); style sac approximately as long as stomach; intestine pigmented (Figure 23D).

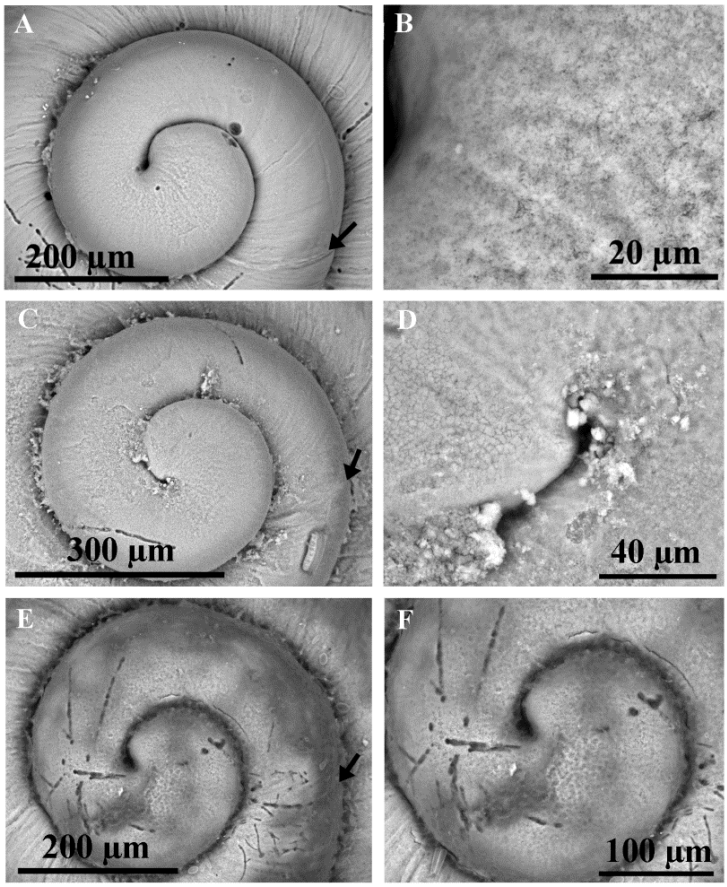


Figure 19. Protoconch details of Majorcan species. **A, B.** *Pseudamnicola (P.) beckmanni* from El Rentador Spring, Deià. **C, D.** *Pseudamnicola (P.) granjaensis* from La Granja, Esporles. **E, F.** *Pseudamnicola (P.) artanensis* from a ditch near Betlem Hermitage, Artá.

Female genitalia with a slightly lobulated albumen gland smaller than the capsule gland (Appendix III: Table 5); bursa copulatrix pyriform with a duct similar or longer in length than bursa copulatrix; renal oviduct lies over bursa copulatrix making one or two loops, it is brown pigmented until insertion of seminal receptacle; elongate seminal receptacle with a short duct; situated on renal oviduct above the insertion of bursal duct (Figure 22G).

Male genitalia with a prostate gland three times longer than wide (Appendix III: Table 6); vas efferens entering the medial-posterior region and vas deferens exiting at the anterior (Figure 22A); triangular penis with a wide base, small pigment patch on distal portion and many folds over the entire surface of internal side (Figure 22D); attached to central region of head.

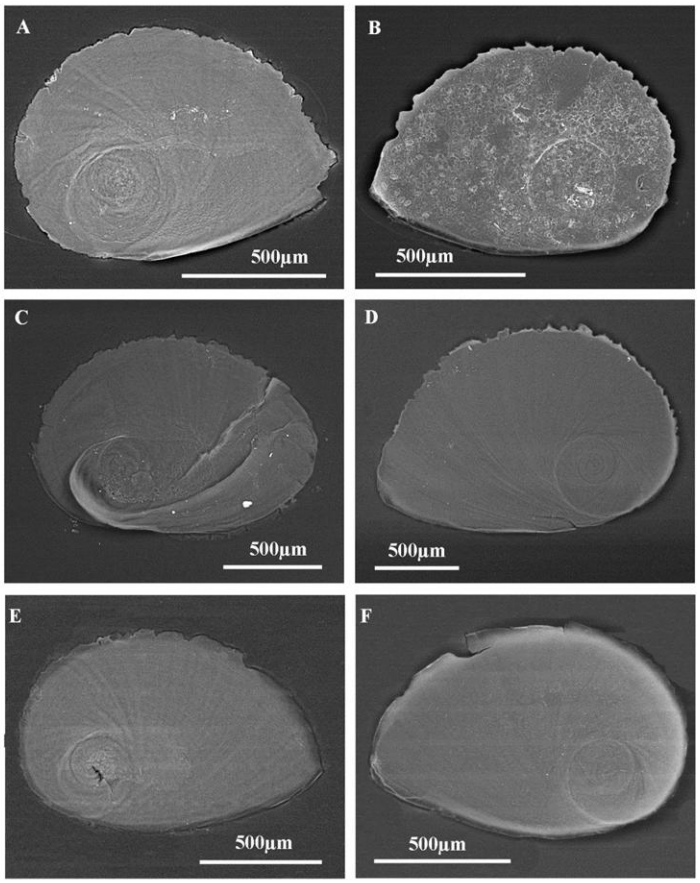


Figure 20. Inner side (left column) and outer side (right column) of the operculum of the following species: **A, B.** *Pseudamnicola (P.) beckmanni* from El Rentador Spring, Deià, Majorca. **C, D.** *Pseudamnicola (P.) granjaensis* from La Granja, Esporles, Majorca. **E, F.** *Pseudamnicola (P.) artanensis* from a ditch near Betlem Hermitage, Artá, Majorca.

Nervous system brown pigmented, darker on ganglia than on connectives and commissures; cerebral ganglia equal in size; supraoesophageal connective approximately nine times longer than suboesophageal (Figure 22J, Appendix III: Table 7); nervous system elongate (mean RPG ratio 0.55); straight oesophagus running beneath nervous system.

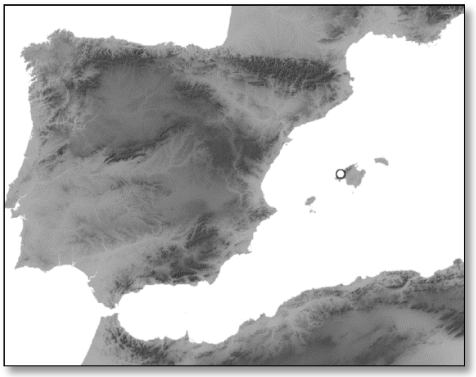
Remarks. This species is mainly distributed along Tramuntana mountain range, in the north of Majorca Island, though it has also been found in a spring in Randa, a village situated in the center of the island. The specimens from this locality seem to bear minor morphological and molecular differences with those from the other mountain localities for this species, which is reflected by the low level of genetic variability: average of 1.2% for COI, 0.53% for 16S and 0.06% for 28S (Appendix IV: Table 1). The remaining Tramuntana populations show even less genetic divergence among them (0.3% for COI, 0.4% for 16S and 0.1% for 28S in mean).

In 1988, Boeters redescribed the species *P. (P.) subproducta* and cited it not only in sites surrounding Banyolas Lake (type locality 1), an Iberian locality in Gerona province, but also in many sites on the islands of Majorca and Minorca. Nevertheless, this author pointed to the existence of marked anatomical variability in characters of shell and female and male genitalia. Boeters (1988) erroneously identified the populations in Majorca and Minorca as '*P. (P.) spirata'* when in fact these populations belong to several different species, namely *P. (P.) beckmanni* (Boeter's figures 55, 76, 82 and 83), *P. (P.) granjaensis* and *P. (P.) artanensis* from Majorca and *P. (P.) meloussensis* (Boeters' figures 53, 54, 77 and 84) from Minorca (see below). Our results show some discrepancies with Boeters's drawings. For instance, focusing on the penis drawings of *P. (P.) subproducta* and *P. (P.) beckmanni* (numbers 75 and 76, respectively in Boeters, 1988), the penis of *P. (P.) beckmanni* seems to be shorter; however, our morphometric study shows the opposite. Moreover, after dissecting some *P. (P.) beckmanni* females from several localities in Majorca, we observe that the bursa copulatrix is not as large as in Boeters' drawings (numbers 82, 83, Boeters, 1988), though it is larger and with a longer duct than the bursa copulatrix of *P. (P.) subproducta* (number 81, Boeters, 1988). Thus, although showing certain similarities in shell habitus (Boeters' schemes 55 and 56), *P. (P.) beckmanni* can be differentiated from *P. (P.) subproducta* by: 1) a shorter penis with a wider base and central attachment area, while the narrower base of the *P. (P.) subproducta* penis is attached behind the right eye; 2) a larger bursa copulatrix with a longer less pigmented duct in *P. (P.) beckmanni* than in *P. (P.) subproducta*; 3) a shorter prostate gland than in *P. (P.) subproducta*; and 4) an elongate nervous system (RPG ratio 0.55), whereas in *P. (P) subproducta*, it is moderately concentrated (RPG ratio 0.45). Furthermore, molecular differences between these two species of 7.7%, 3% and 0.9% for COI, 16S and 28S, respectively confirm that they are different taxa.

Pseudamnicola (Pseudamnicola) granjaensis Glöer and Zettler, 2007

Type locality

La Granja, Majorca, Balearic Islands, 39.671º N, 2.559º E (Glöer and Zettler, 2007).



Type material

Holotype (ZMH 51002) and three paratypes (ZMH 51003) in Zoologischen Museum, Hamburg; three in Glöer's collection, 41 in Zettler's collection and four in Beckmann's collection.

Localities

This species is only known from the type locality. After exploring the area of La Granja, we found the species in one fountain near the exit of the palace La Granja, Esporles, Majorca (Appendix II).

Material examined for morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Tables 1-7) taken from specimens collected from a fountain in La Granja, Esporles, Majorca. Only 10 specimens were collected. The two males and the female studied anatomically were collected in April 2008.

New diagnosis

Shell with yellowish periostracum; last body whorl occupying about two-thirds of shell length; central tooth of radula with five tapered lateral cusps; long gastric caecum; black pigmentation on intestine; large pyriform bursa copulatrix; elongate seminal receptacle; renal oviduct black pigmented until insertion of seminal receptacle; penis gradually tapering with a small patch of pigmentation on the tip and attachment area central; supraoesophageal connective about six times longer than suboesophageal one; nervous system elongate (RPG ratio 0.60).

Description

Shell ovate-conic, yellowish (Figures 18B, E, H) with 4.75-5.50 spire whorls and a height of approximately 3.50-5 mm; protoconch about 500 µm in width with 1.4 whorls and a nucleus around 120 µm in length; protoconch microsculpture grooved (Figures 19C, D); convex whorls and deep sutures; inner lip of aperture thicker than outer one; the edge of peristome is straight (Figure 18E).

Operculum with 3.5 whorls and an oval muscle attachment area located near the nucleus (Figures 20C, D; Appendix III: Table 2).

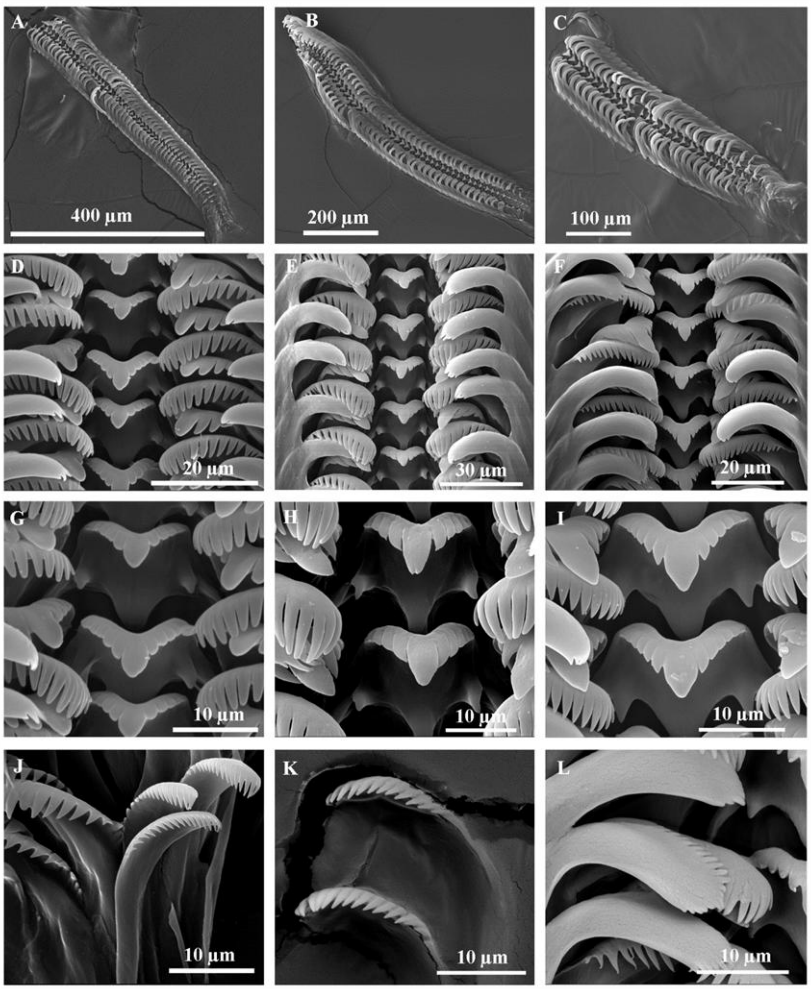


Figure 21. General view of radula (first row), rows of teeth of the radula (second row), central teeth (third row) and detail of outer marginal teeth (fourth row) of the Majorcan species: **A, D, G, J.** *Pseudamnicola (P.) beckmanni* from El Rentador Spring, Deià. **B, E, H, K.** *Pseudamnicola (P.) granjaensis* from La Granja, Esporles**. C, F, I, L.** *Pseudamnicola (P.) artanensis* from a ditch near Betlem Hermitage, Artá.

Radula length intermediate (20% of total shell length) and approximately six times longer than wide (Figure 21B; Appendix III: Table 3); around 55 rows of teeth; central tooth with a tapered median cusp and five pointed lateral cusps (Figures 21E, H); lateral teeth with three tapered cusps shorter than the central one; inner marginal teeth bear approximately 15 tapered cusps, shortening towards the base of tooth; outer marginal teeth also with about 15 tapered cusps (Figure 21K).

Pigmentation and anatomy. Head dark brown pigmented from snout to neck (Figure 22E); the pigmentation is lighter on neck; tentacles with medial longitudinal stripe lacking pigment; no pigment on ocular lobes; snout as long as wide, with a medium distal lobation; foot of intermediate length with pigmentation on dorsal region. Ctenidium in the middle region of pallial cavity with 20-25 gill filaments longer than wide; osphradium of intermediate width under central gill filaments (Figure 23B, Appendix III: Table 4). Stomach slightly longer than wide and posterior chamber slightly larger than the anterior one (Figure 23E, Appendix III: Table 4); long gastric caecum; style sac longer than wide and surrounded by intestine which features a small stripe of brown pigment.

Female genitalia with a pallial oviduct containing a capsule gland and an albumen gland of nearly equal size (Appendix III: Table 5); large pyriform bursa copulatrix with a duct about two times shorter than bursa length; renal oviduct black pigmented until point of insertion of seminal receptacle; elongate seminal receptacle laying on renal oviduct slightly above the point where the bursal duct joins the renal oviduct (Figure 22H).

Male genitalia contains a prostate gland about three times longer than wide (Figure 22B, Appendix III: Table 6); penis gradually tapering with folds along the entire surface and a path of brown pigment on its distal region; the base is attached to central area of the head (Figure 22E).

Nervous system dark brown pigmented; cerebral ganglia equal in size and darker than other ganglia and connectives; supraoesophageal connective approximately six times longer than suboesophageal one (Figure 22K; Appendix III: Table 7). Mean RPG ratio is 0.60 (elongate).

Remarks. Boeters' monograph (1988) recorded the species *P. (P.) subproducta* in Esporles, ("Font d'en Bassina"). Nevertheless, we did not find that species in Esporles or its surroundings; rather, we find *P. (P.) beckmanni* and *P. (P.) granjaensis.* In addition to being genetically distant (7.9%, 2.8% and 0.8% for COI, 16S and 28S, respectively), *P. (P.) subproducta* and *P. (P.) granjaensis* can be further differentiated by several features: longer and more conic shells in *P. (P.) granjaensis*; larger penis presenting many folds along its inner surface in *P. (P.) subproducta*; longer bursa copulatrix and seminal receptacle in *P. (P.) granjaensis*;

and a moderately concentrated nervous system in *P. (P.) subproducta*, while elongate in *P. (P.) granjaensis.*

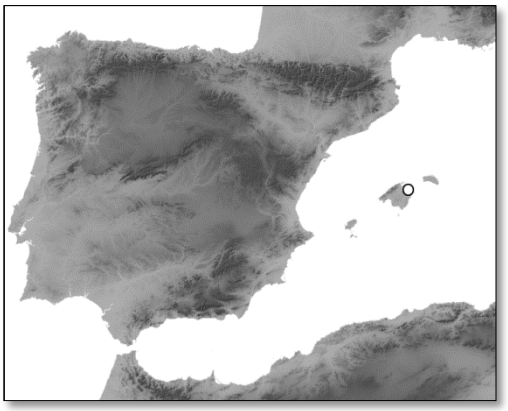
The species *P. (P.) beckmanni* is also found in the gardens of La Granja and is genetically close to *P. (P.) granjaensis* (0.5% for COI, 0.3% for 16S and 0.1% for 28S). However, some anatomical and morphological differences exist between them. *P. (P.) granjaensis* is distinguishable from *P. (P.) beckmanni* by several features: a longer shell (mean of 4.28 mm, Appendix III: Table 1; this measurement is greater than 2.9-3.0 mm, the length published in the original description of Glöer and Zettler, 2007); a larger bursa copulatrix (Appendix III: Table 5), but a proportionally shorter bursal duct; a larger prostate gland and longer, narrower penis (Appendix III: Table 6); and longer nervous system connectives, and therefore, a higher RPG ratio.

Although the conic shape and long size of *P. (P.) granjaensis* shells are two features more commonly associated with the subgenus *P. (Corrosella)*, both the molecular data and internal anatomy confirm this species as belonging to *P. (Pseudamnicola).*

***Pseudamnicola (Pseudamnicola) artanensis* Altaba, 2007**

Type locality

Spring near Betlem Hermitage, Artá, Majorca, Balearic Islands (Altaba, 2007).



Type material

Holotype (CRA-6085-1) and 40 paratypes (CRA-6085) in Altaba's personal collection.

Localities

This species has only been found at the type locality.

Material examined for morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Tables 1-7) correspond to specimens collected in April 2008 from a spring near Betlem Hermitage, Artá, Majorca.

New diagnosis

Shell slender, peristome with inner lip thicker than outer one; radula with six pointed lateral cusps in central tooth and three in lateral tooth; intestine pigmented; female genitalia with a pyriform J-shaped bursa copulatrix and an elongate seminal receptacle; long bursal duct proportional to bursa length; triangular penis with wide base, a small patch of black pigment on distal region and many folds over the entire inner surface; nervous system brown pigmented; supraoesophageal connective around five times longer than suboesophageal.

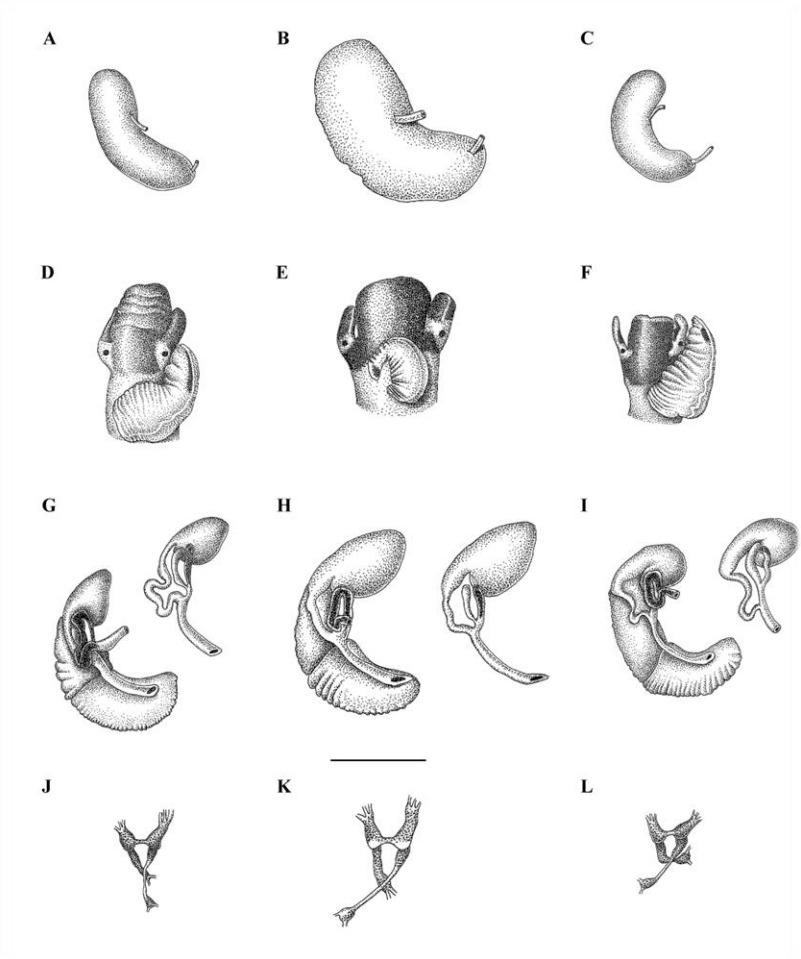


Figure 22. Prostate gland (first row), head and penis of a male (second row), female genitalia with details of the bursa copulatrix and seminal receptacle (third row) and nervous system (fourth row) of the Majorcan species: **A, D, G, J.** *Pseudamnicola (P.) beckmanni* from El Rentador Spring, Deià. **B, E, H, K.** *Pseudamnicola (P.) granjaensis* from La Granja, Esporles. **C, F, I, L.** *Pseudamnicola (P.) artanensis* from a ditch near Betlem Hermitage, Artá.

Description

Shell yellowish periostracum, with 4-4.5 spire whorls (Figures 18C, F, I, Appendix III: Table 1), height of between 3.00-3.50 mm; protoconch approximately 350 μm wide, with 1.3 whorls and a nucleus about 150 μm long; protoconch microsculpture granulated (Figures 19E, F); body whorl about two-thirds of total length; peristome with thin outer lip and thick inner lip which partially hides the umbilicus; edge of peristome simple and straight (Figure 18F).

Operculum translucent, with approximately 2.5 spire whorls; internal side bears a convex edge and oval muscle attachment near nucleus (Figures 20E, F, Appendix III: Table 2).

Radula size small (<15% of total shell length), four times longer than wide, and with around 35 rows of teeth (Figure 21C, Appendix III: Table 3); trapezoidal central tooth with a tongue-shaped median cusp and six pointed lateral cusps (Figures 21F, I); lateral teeth with three tapered lateral cusps and a median cusp larger than lateral cusps; inner and outer marginal teeth with around 20 cusps (Figure 21L).

Pigmentation and anatomy. Head with dark brown pigment all over its surface except on the edge of snout, around the ocular area and along a medial longitudinal stripe of tentacles (Figure 22F); dorsal region of foot pigmented; pigment on neck lighter than on the head; foot of intermediate size with anterior edge indented. Ctenidium with 17-20 well-developed gill filaments occupying most of the pallial cavity; osphradium located in opposite middle of ctenidium and two times longer than wide (Figure 23C, Appendix III: Table 4). Stomach with a posterior chamber larger than anterior chamber and style sac shorter than stomach, longer than wide (Appendix III: Table 4); the portion of the intestine surrounding style sac dark pigmented (Figure 23F).

Female genitalia with a capsule gland slightly longer than albumen gland (Appendix III: Table 5); bursa copulatrix pyriform J-shaped with a long bursal duct almost as long as bursa copulatrix; elongate seminal receptacle attached a little above the distal end of renal oviduct; renal oviduct pigmented making one or two loops, later it continues straight from the loop until insertion of the bursal duct; pigmentation along renal oviduct decreases from loop to seminal receptacle (Figure 22I).

Male genitalia with a prostate gland about three times longer than wide (Appendix III: Table 6); vas efferens entering the medial-posterior region and vas deferens exiting at the anterior (Figure 22C); triangular penis with a wide base, small pigmented patch on the distal section and many folds over the entire inner surface (Figure 22F); attached to central region of head; with a highly curved penial duct along its right side.

Nervous system brown pigmented, darker on ganglia than on connectives and commissures; cerebral ganglia equal in size; supraoesophageal connective five times longer than suboesophageal one (Figure 22L, Appendix III: Table 7); RPG ratio 0.50 (moderately concentrated); straight oesophagus running beneath nervous system.

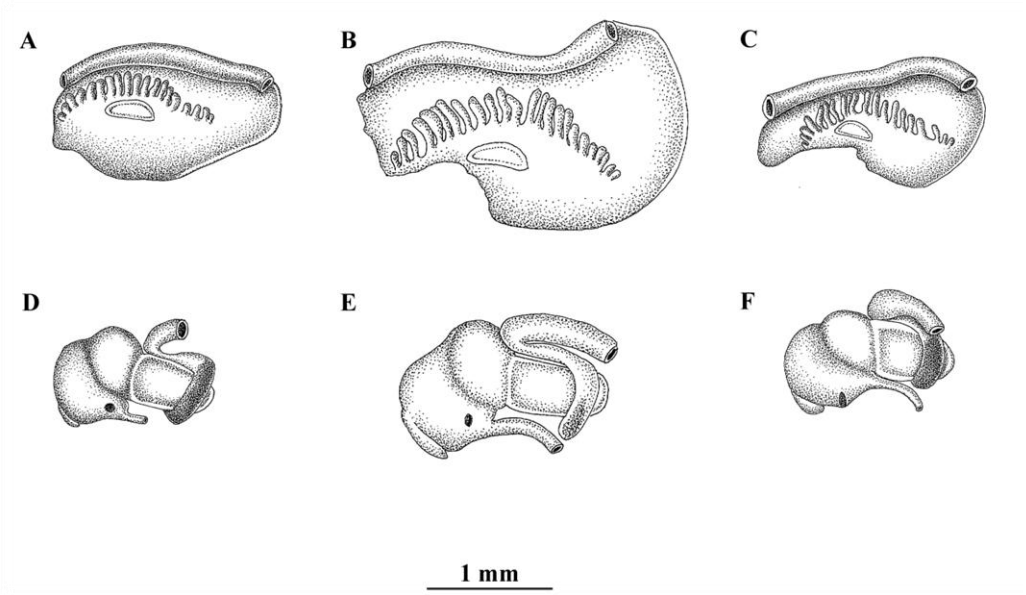
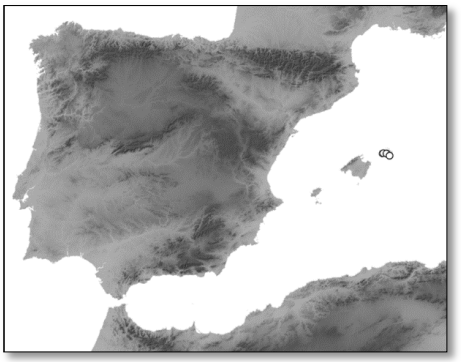


Figure 23. Ctenidium, osphradium and rectum (first row) and stomach (second row) of the Majorcan species: **A, D.** *Pseudamnicola (P.) beckmanni* from El Rentador Spring, Deià. **B, E.** *Pseudamnicola (P.) granjaensis* from La Granja, Esporles. **C, F.** *Pseudamnicola (P.) artanensis* from a ditch near Betlem Hermitage, Artá.

Remarks. Until now, this species was only known from the type locality. It was originally assigned to the species *P. (P.) subproducta* by Boeters (1988), although Altaba later described it as a new species (Altaba, 2007). In Altaba's description, the penis seems to be longer and narrower than the sizes found in our observations. This difference may be because our specimens were fixed directly in ethanol without first being anesthetized, and thus, the penis may have contracted during the process. Nevertheless, differences between *P. (P.) artanensis* and *P. (P.) subproducta* are based on a combination of characters. Despite bearing the same number of spire whorls, the shell of *P. (P.) artanensis* is longer and narrower; the bursa copulatrix, bursal duct and seminal receptacle of *P. (P.) artanensis* are also longer. However, male genitalia (i.e. prostate gland and penis) present similar dimensions in both species. Additionally, the genetic distances between them are 7.3% for COI, 3.1% for 16S and 1.8% for 28S, may indicate that they are separate species.

Relative to the other species from Majorca, *P. (P.) artanensis* can be distinguished by having an intermediate shell size between the shell sizes of *P. (P.) beckmanni* and *P. (P.) granjaensis* (Appendix III: Table 1), a greater number of cusps in the central, inner and outer marginal teeth of the radula (Appendix III: Table 3), the longest bursal duct, the largest penis and a similar RPG ratio as *P. (P.) beckmanni* but lower than *P. (P.) granjaensis*.

Pseudamnicola (Pseudamnicola) meloussensis Altaba, 2007



Type locality

Stream at Macarella Creek, Minorca, Balearic Islands (Altaba, 2007).

Type material

Holotype (CRA- -1) and paratypes (CRA-) in Altaba's personal collection.

Other populations studied

To assess the localities from Minorca published by Boeters (1988), we returned to the island and found most of them destroyed mainly from the construction of pipes and irrigation systems. Nevertheless, localities in the south, including the type locality, were conserved and hence, they are the populations examined in this study (Appendix II).

Specimens examined for morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Tables 1-7) were made in male and female specimens collected from a stream at Macarella Creek (type locality), Minorca in December 2007.

New diagnosis

Shell with a bulging inflated body whorl occupying around four-fifths of shell length; central radular tooth with five lateral cusps decreasing in size; bursa copulatrix pyriform and bursal duct about 75% of the length of bursa copulatrix; elongate seminal receptacle; black pigmented renal oviduct with pigment fading from loop to insertion of seminal receptacle; penis shape between triangular and tapered with rounded tip, small patch of pigmentation on distal section and folds

over entire inner surface; in most of the cases, with a constriction in the beginning of the distal region; brown pigmented nervous system with darker cerebral ganglia, and supraoesophageal connective more than eight times longer than suboesophageal one.

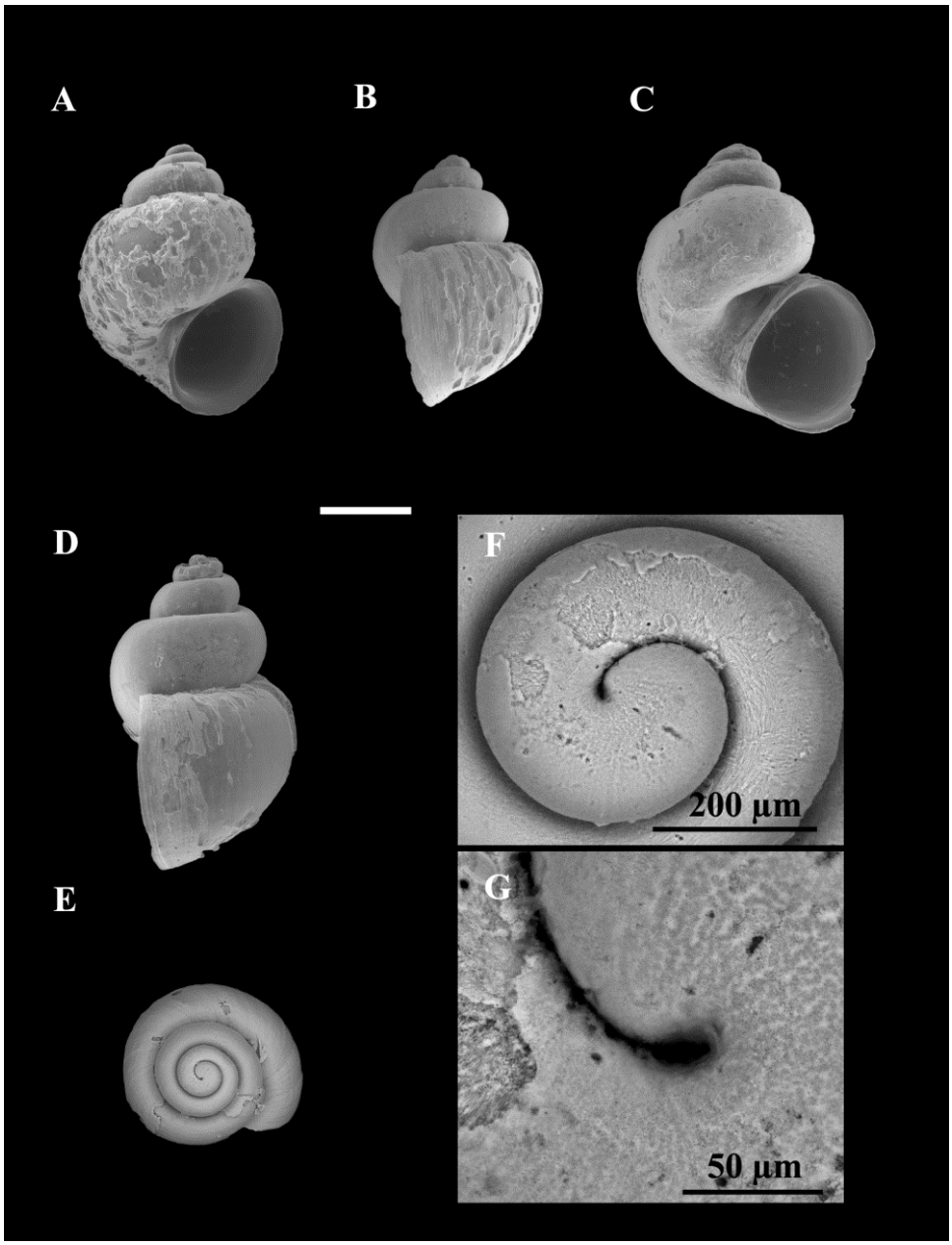


Figure 24. Shells of *Pseudamnicola (P.) meloussensis* from a stream at Macarella Creek, Minorca. **A-D.** Frontal and lateral views of the observed morphotypes. **E-G.** Protoconch and details of protoconch microsculpture.

Description

Shell ovate-conic, yellowish periostracum with 3.75-4.50 spire whorls and a height between 2.5-3.5 mm (Figures 24A-C, Appendix III: Table 1); body whorl well-developed, about four-fifths of shell length; deep suture and convex spire whorls; protoconch with approximately 1.3 whorls; protoconch and nucleus width around 450 µm and 125 µm, respectively (Figures 24E, F); protoconch microsculpture granulated (Figure 24G); oval aperture with thin outer lip and slightly thicker inner lip; wide umbilicus; edge of peristome straight (Figure 24D).

Operculum with around 2.5 spire whorls and an oval muscle attachment area near the nucleus (Figures 25A, B, Appendix III: Table 2).

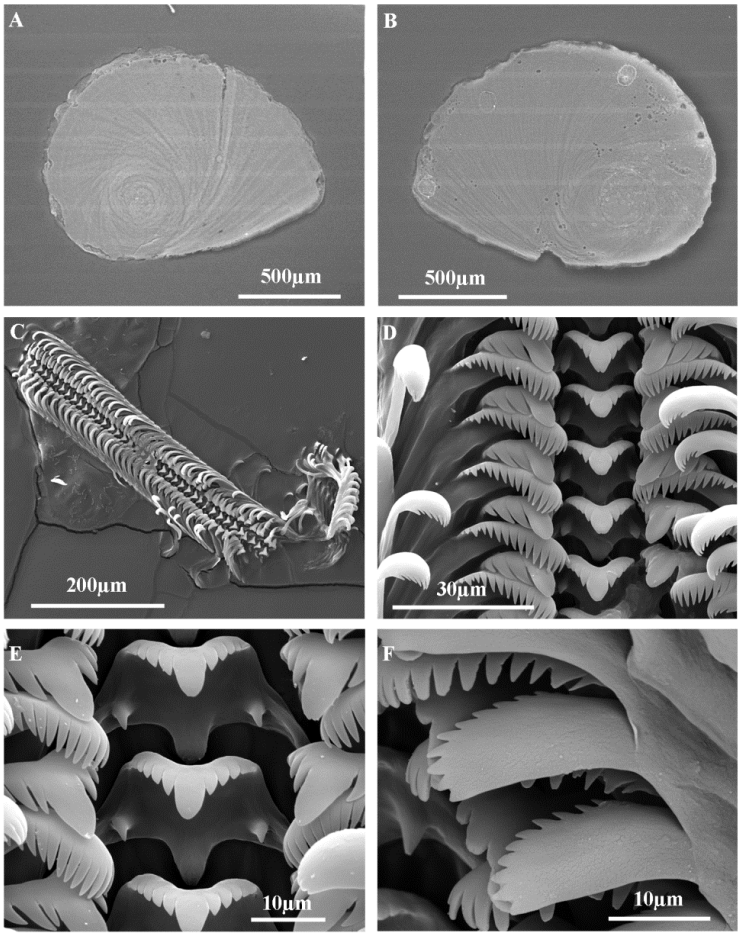


Figure 25. Operculum and radula of *Pseudamnicola (P.) meloussensis* from a stream at Macarella Creek, Minorca. **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central teeth. **F.** Outer marginal teeth.

Radula length medium (25%) relative to maximum shell dimension (Figure 25C, Appendix III: Table 3); with approximately 50 rows of teeth; central tooth with a large median cusp followed on each side by five small cusps decreasing in size (Figures 25D, E); lateral teeth with three sharp lateral cusps; inner marginal teeth with approximately 18 tapered cusps and outer marginal teeth with around 12 tapered cusps smaller than inner marginal cusps (Figure 25F).

Pigmentation and anatomy. Head brown pigmented, less pigmentation on tentacles and neck; lack of pigmentation on edge of snout and ocular area (Figure 26F); foot intermediate in size and with dark brown pigment on its dorsal side. Ctenidium well-developed with 18–20 gill filaments longer than wide, occupying the middle section of pallial cavity; osphradium in opposite middle region of ctenidium (Figure 26C, Appendix III: Table 4). Stomach slightly longer than wide (Appendix III: Table 4); oesophagus and intestine lack pigmentation (Figure 26E); rectum S-shaped in pallial cavity.

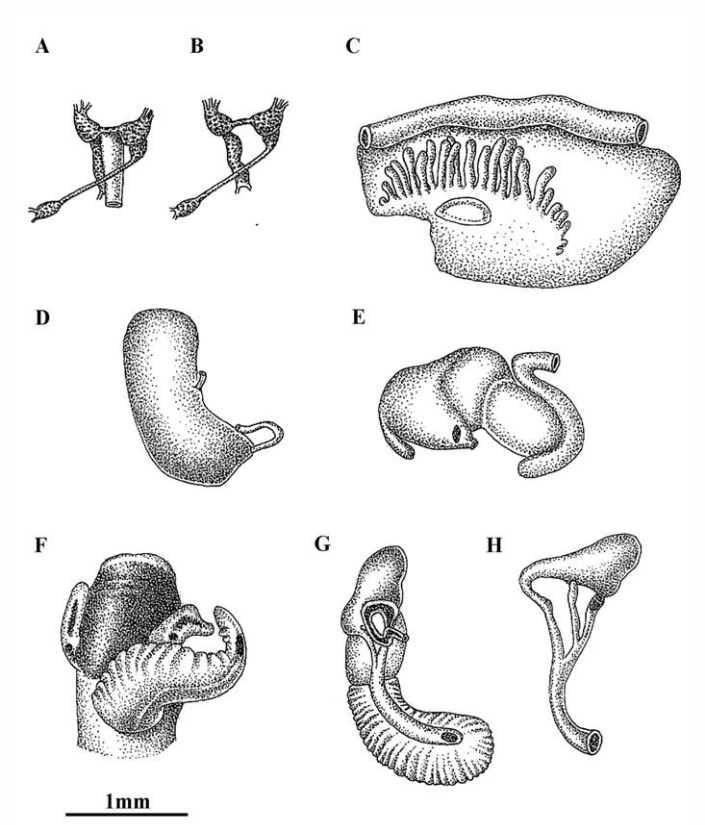


Figure 26. Anatomy of *Pseudamnicola (P.) meloussensis* from a stream at Macarella Creek, Minorca. **A, B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Prostate gland. **E.** Stomach. **F.** Head and penis of a male. **G.** Female genitalia. **H.** Bursa copulatrix and seminal receptacle.

Female genitalia with a capsule gland longer than albumen gland (Figure 26G, Appendix III: Table 5); bursa copulatrix pyriform and bursal duct about 75% of the length of bursa copulatrix; elongate seminal receptacle without duct, situated on renal oviduct above the insertion of bursal duct; black pigmented renal oviduct, pigment fading from loop to insertion of seminal receptacle; renal oviduct lies over bursa copulatrix making one or two loops (Figure 26H).

Male genitalia with a bean-shaped prostate gland approximately four times longer than wide (Figure 26D, Appendix III: Table 6); penis shape between triangular and tapered with rounded tip and folds over entire surface, folds thicker near the tip; it is attached to the central area of the head; in most of the cases, there is a constriction in the beginning of the distal region; small patch of pigmentation on distal section of penis (Figure 26F); penial duct runs straight (although in some specimens wavy), near the outer edge of the penis.

Nervous system with darker cerebral ganglia than connectives and commissures; cerebral ganglia approximately equal in size; supraoesophageal connective eight times longer than suboesophageal one (Figure 26B, Appendix III: Table 7); RPG ratio 0.60 (elongate); oesophagus runs straight underneath nervous system (Figure 26A).

Remarks. The type locality presents two shell morphotypes, one that is smaller with a more roundish aperture (Figure 24A) and the other that has a larger last body whorl and wider umbilicus and aperture (Figure 24C). The first morphotype describes the one in the original description (Altaba, 2007). However, both morphotypes were figured by Boeters (1988, Figures. 53-54) from Sant Joan de Carbonell, Minorca. Anatomically, only minor differences are found between morphotypes, mainly in penis features: the penis of the second morphotype is more slender and tapered. The greatest genetic distance among individuals of this population is 2% for COI, which seems to be insufficient for considering them different species.

The general shape of the shell and penis for the other examined localities is the one with a smaller shell and less tapered penis. However, the penis tip is less pointed than that of Boeters' descriptions for specimens from Minorca (Figure 77 of Boeters, 1988). The female genitalia are similar to Figure 84 of Boeters (1988). There is low anatomical variability within and between populations; however, a certain level of genetic divergence exists between them. Specimens collected from Sen Penyes are genetically the most divergent of the Minorcan specimens (the greatest genetic distance observed was at 2.6% for COI). This locality is geographically the most isolated compared to the other localities, which may decrease gene flow.

In 1988, Boeters assigned some populations from Minorca to *P. (P.) subproducta* claiming that some intraspecific variability existed for this group. However, despite sharing shell dimensions and number of spire whorls, we conclude that the studied populations from Minorca belong to a different species group, namely *P. (P.) meloussensis*. This species differs from *P. (P.) subproducta* in a number of characters as follows: 1) longer stomach style sac; 2) larger capsule gland and bursa copulatrix; 3) longer penis; 4) elongate nervous system (RPG ratio of 0.60); and 5) genetically, having divergences of 5.6% for COI, 1.5% for 16S and 1.1% for 28S.

Pseudamnicola (Pseudamnicola) subproducta (Paladilhe, 1869)

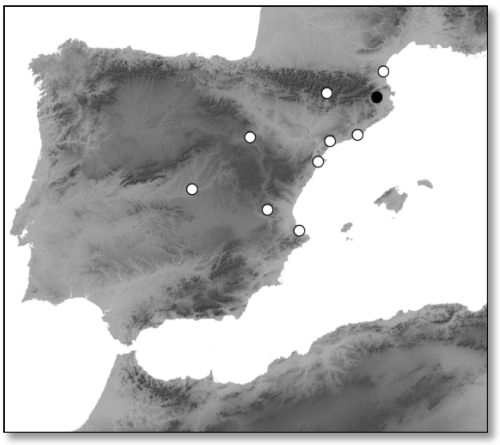
Amnicola spirata Paladilhe, 1869, Nouv. Miscel. Malac., p.108, pl. v, fig. 10-11. (feb. 1869).

Amnicola subproducta Paladilhe, 1869, Nouv. Miscel. Malac., p.140; note additionelle (feb 1869). nom. nov. pro *Amnicola spirata*. *Pseudamnicola (Pseudamnicola) spirata* (Paladilhe): Boeters 1988.

Pseudamnicola (Pseudamnicola) subproducta (Paladilhe): Soler, Moreno, Araujo and Ramos, 2006. *Graellsia*, 62 (num. extraord.): 212. n. comb.

Type locality

[1] Surrounding areas of Banyoles (Catalonia) and [2] near Salses (eastern Pyrenees) (Paladilhe, 1869).



According to Boeters (1988), the syntypes from locality [1] Gerona, Banyoles, belong to this species and were collected from a ditch on the north end of the lake, fed by a spring in a brick culvert and a stream that, after about 100 meters, flows into Banyoles Lake.

Type material

Not found.

Other populations studied

After several visits to Banyolas Lake, we did not find *P. (P.) subproducta* but only *Potamopyrgus antipodarum*. However, a few specimens from a single population found in the area surrounding Salses (Font Estramar, near Perpignan, France) were collected and studied. Based on these specimens, we confirm that these belong to *P. (P.) subproducta*. In addition, this species was found in several localities in the northeastern region of the Iberian Peninsula (see Appendix II).

Specimens examined for morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Tables 1-7) were taken from male and female specimens from Baltasar Ullal and Ontígola Lagoon in the provinces of Tarragona and Madrid (Spain), respectively. These two localities were examined because the species is very abundant, and many specimens per population could be obtained.

New diagnosis

Shell yellowish with a body whorl occupying three-fourths of shell length and a deep suture between whorls; protoconch microsculpture grooved or granulated; central radular tooth with five lateral cusps; style sac surrounded by an intestine, usually pigmented; pyriform bursa copulatrix; elongate seminal receptacle without duct; triangular penis with a small black patch of pigmentation in its distal region and folds over the entire surface; nervous system brown pigmented with supraoesophageal connective about seven times longer than suboesophageal.

Description

Shell ovate-conic with 3.25-4.50 spire whorls, with a height of between 2.0-3.75 mm (Figures 27A-C, Appendix III: Table 1); periostracum yellowish; protoconch approximately 400 μm wide with 1.5 whorls and a nucleus around 100 μm long (Figures 27E, F); protoconch microsculpture either grooved or granulated, more intense on apex (Figures 27G, H); body whorl about three-fourths the total length; teleoconch whorls convex with a deep suture; aperture with an inner lip thicker than outer lip; umbilicus marked; peristome margin simple, straight (Figure 27D).

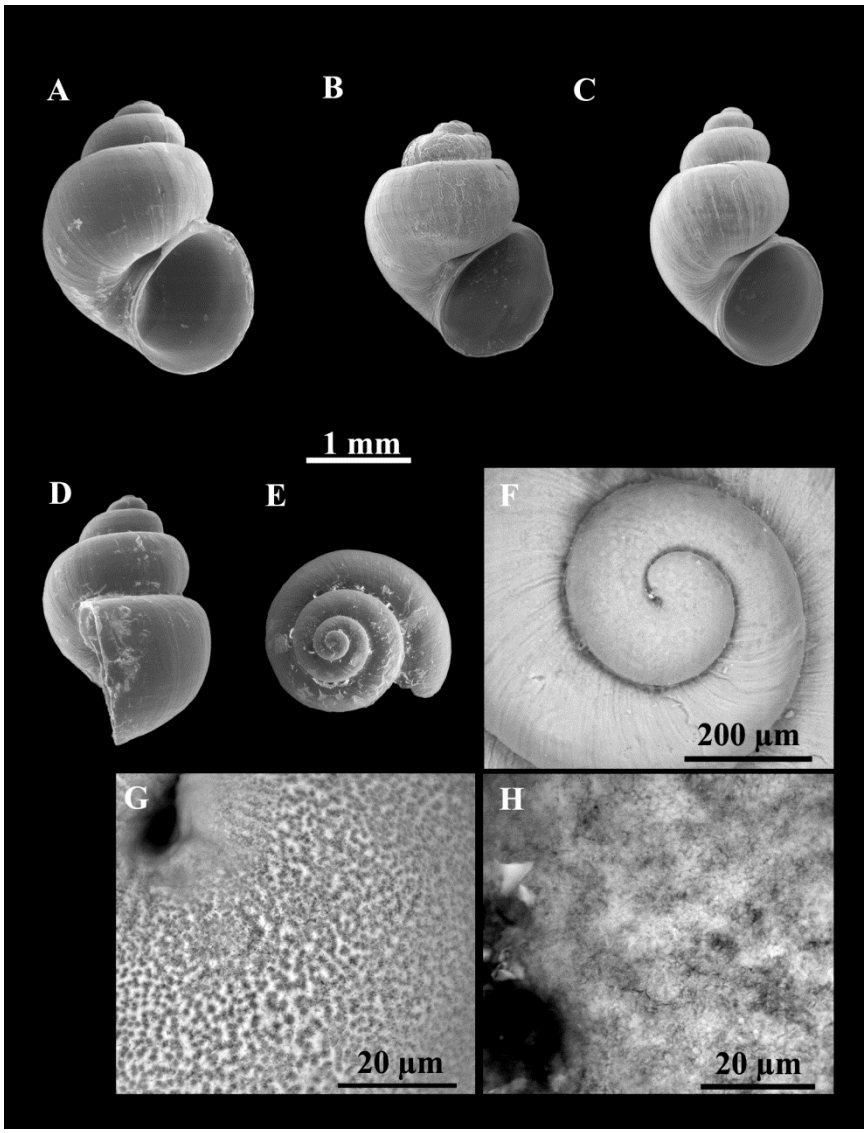


Figure 27. Shells of *Pseudamnicola (P.) subproducta*. **A, D-E, F, H.** Shells from Baltasar Ullal, Tarragona, Spain. **B.** Shell from Font Estramar, Perpignan, France. **C.** Shell from Ontígola Lagoon, Madrid, Spain. **E-F, H.** Protoconch and microsculpture of shell from Baltasar Ullal, Tarragona, Spain. **G.** Microsculpture of shell from Ontígola Lagoon, Madrid, Spain.

Operculum with approximately 2.5 spire whorls on internal side; oval muscular attachment mark appears near the nucleus (Figures 28A, B, Appendix III: Table 2).

Radula approximately five times longer than wide (Figure 28C, Appendix III: Table 3) and intermediate in size (17% of total shell length); contains around 35 rows of teeth; central tooth with five pointed lateral cusps on each side of a larger central cusp (Figures 28D,E); lateral teeth contain a tongue-shaped central cusp and three

lateral cusps; inner and outer marginal teeth both have 20 cusps each, which become smaller towards the tooth base (Figure 28F).

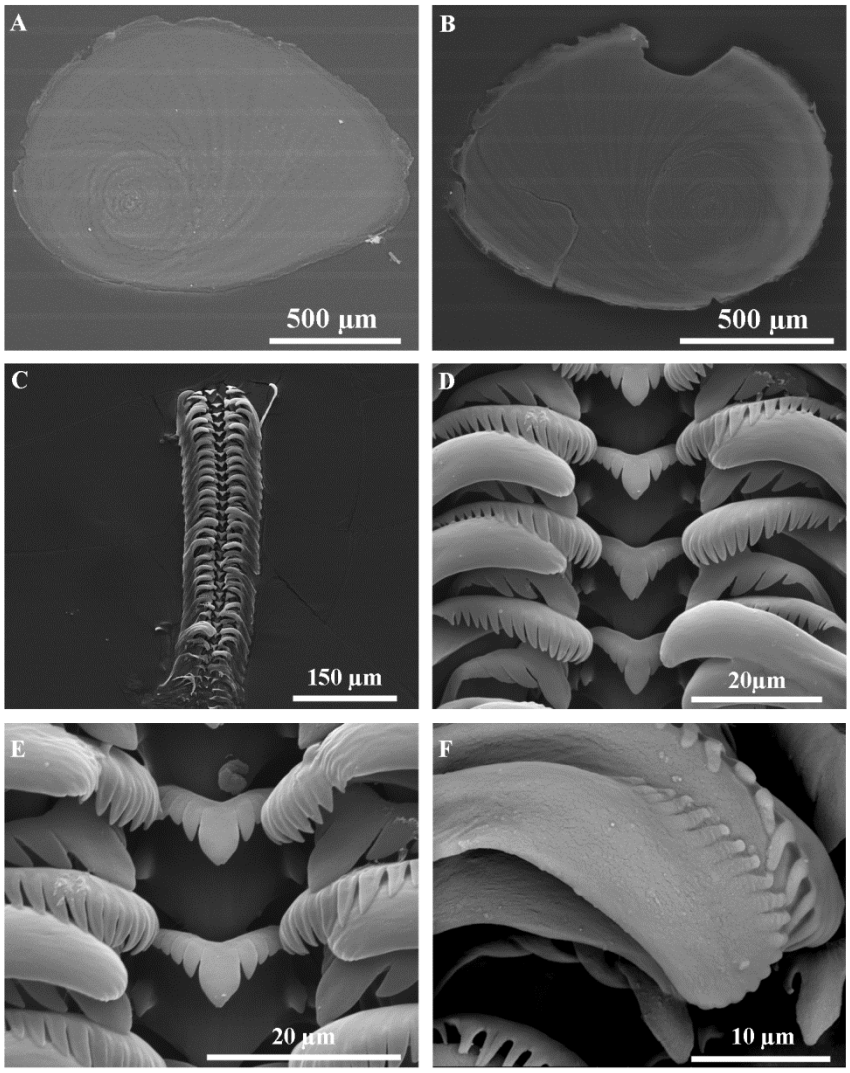


Figure 28. Operculum and radula of *Pseudamnicola (P.) subproducta* from Baltasar Ullal, Tarragona, Spain. **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central teeth. **F.** Details of outer marginal teeth.

Pigmentation and anatomy. Head and tentacles pigmented dark brown, but pigment absent in ocular lobe regions, edge of snout and along a longitudinal stripe on tentacles (Figure 29F); foot intermediate in size with dark brown pigmentation on its dorsal side. Ctenidium in the middle of the pallial cavity and contains around 20 gill filaments longer than wide; osphradium opposite middle section of ctenidium

(Figure 29C, Appendix III: Table 4). Stomach and style sac slightly longer than wide (Appendix III: Table 4); its anterior region being surrounded by a pigmented intestine (Figure 29E); rectum slightly S-shaped in pallial cavity.

Female genitalia with a pallial oviduct containing a capsule gland approximately as long as the albumen gland (Figure 29G, Appendix III: Table 5); pyriform bursa copulatrix with a duct usually longer than bursa length; renal oviduct black pigmented until loop; elongate seminal receptacle with a short duct inserted slightly above the point where the bursal duct joins the renal oviduct (Figure 29H).

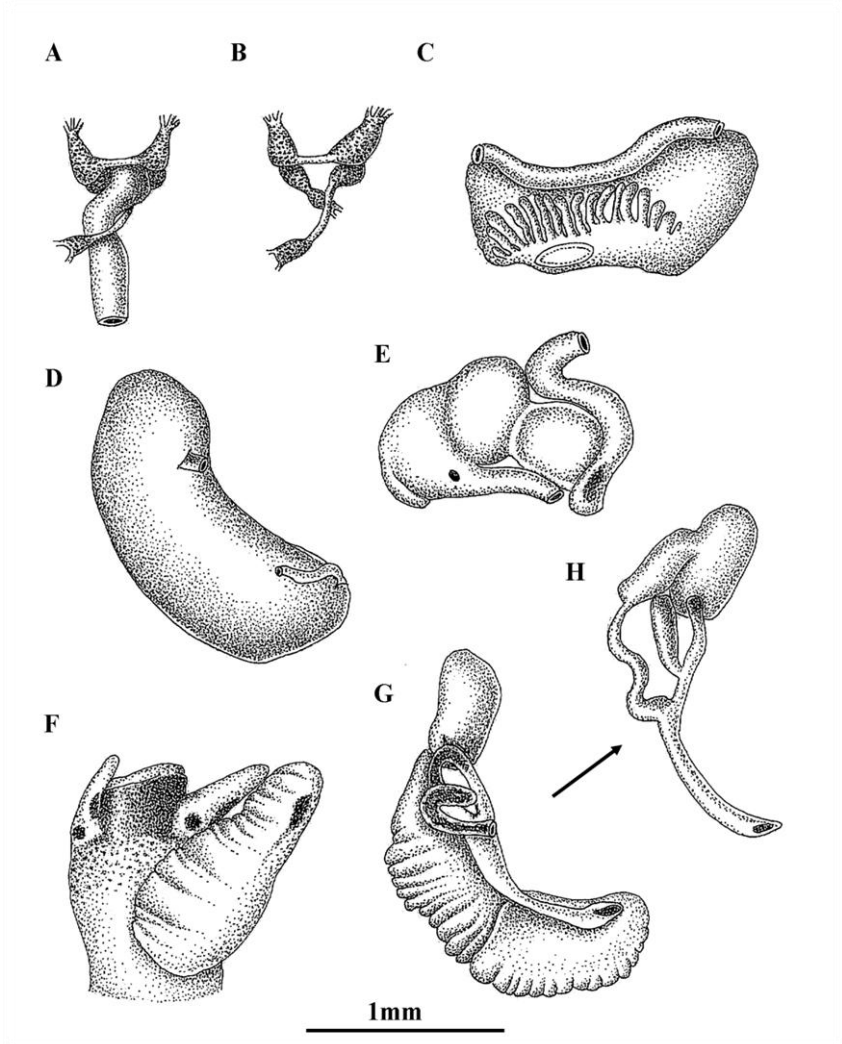


Figure 29. Anatomy of *Pseudamnicola (P.) subproducta* from Baltasar Ullal, Tarragona, Spain. **A, B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Prostate gland. **E.** Stomach. **F.** Head and penis of a male. **G.** Female genitalia. **H.** Bursa copulatrix and seminal receptacle.

Male genitalia bear a prostate gland about three times longer than wide (Figure 29D, Appendix III: Table 6); penis triangular with rounded tip and a pacth of brown pigment, which varies in size, on its distal region; folds over its entire surface; the base is attached to central area of the head (Figure 29F).

Nervous system brown pigmented, but ganglia darker than connectives and commissures; cerebral ganglia equal in size; supraoesophageal connective approximately seven times longer than suboesophageal (Figures 29A, B; Appendix III: Table 7). Mean RPG ratio 0.45 (moderately concentrated).

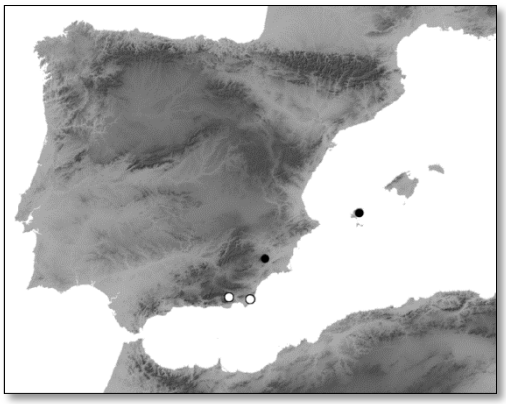
Remarks. The species *P. (P.) subproducta* has been cited for the Iberian Peninsula, Southern France and the islands of Majorca and Minorca (Boeters, 1988). However, after studying populations from these three regions, we conclude that the species occurs only in the Iberian Peninsula and Southern France, and that the populations from the islands actually belong to the species *P. (P.) beckmanni*, *P. (P.) granjaensis*, *P. (P.) artanensis* and *P. (P.) meloussensis*. Although all of these species share features with *P. (P.) subproducta*, such as an ovate-conic shell, a triangular penis, a pyriform bursa copulatrix and an elongate seminal receptacle, *P. (P.) subproducta* can be differentiated from the others by its smaller bursa copulatrix, shorter bursal duct and moderately concentrated nervous system (RPG ratio around 0.45) and, in most of the examined specimens, an oesophagus that moves from side to side underneath the nervous system.

Within the Iberian Peninsula, this species has a wide, but non-contiguous, distribution range, and therefore, some populations are isolated. This fragmented distribution may lend itself to intraspecific variation and account for the observation that individuals from Font Estramar show a more depressed shell (Figure 27B) and smaller penis, while those from Baltasar Ullal have larger shells, yet most of their internal organs are smaller than in specimens from Ontígola Lagoon. Furthermore, protoconch microsculpture is grooved in Baltasar Ullal shells and granulated in Ontígola Lagoon ones (Figures 27G, H). Genetically, the greatest divergences exist between a spring in Les Borges (Catalonia) and Ontígola Lagoon localities, reaching as high as 3.2% for COI.

Pseudamnicola (Pseudamnicola) gasulli **Boeters, 1981**

Type locality

Hotel Fenicia, Santa Eulalia, Ibiza (Boeters, 1981).



Type material

Holotype SMF 253582; paratypes SMF 253583/1, RMNH, GAS and BOE 856 and 929.

Other populations studied

After several visits to the area surrounding the Hotel Fenicia (type locality), we did not find the species either in the Santa Eulalia River or in a wetland situated near the hotel. We also did not find this species in the Rambla locality from Puerto de la Cadena in Murcia province, Spain (Suárez y Vidal-Abarca, 1983; Boeters, 1988: BOE 1235). However, this species was eventually found and collected in several locations from Almería province (Spain) (Appendix II).

Material examined for morphometry

Shell, anatomical, operculum and radular measurements were taken from specimens from Retamar Rambla, a stream at Rambla de los Yesos (Alboloduy) and from a stream at Barranco de las Negras, both of which are located in Almería province (Spain). Both males and females were dissected and measured (Appendix III: Tables 1-7).

Diagnosis

Shell ovate-conic, yellowish with body whorl occupying two-thirds of shell length; whorls convex with deep sutures; protoconch microsculpture granulated; central radular tooth formula 4(5)-C-4(5); pigmented intestine; pyriform bursa copulatrix with a duct approximately one and a half times longer than bursal length; absence of seminal receptacle; prostate gland twice as long as wide; strap-like penis dark pigmented, with a narrow base attached to central area of head; nervous system brown pigmented with supraoesophageal connective about five times longer than suboesophageal.

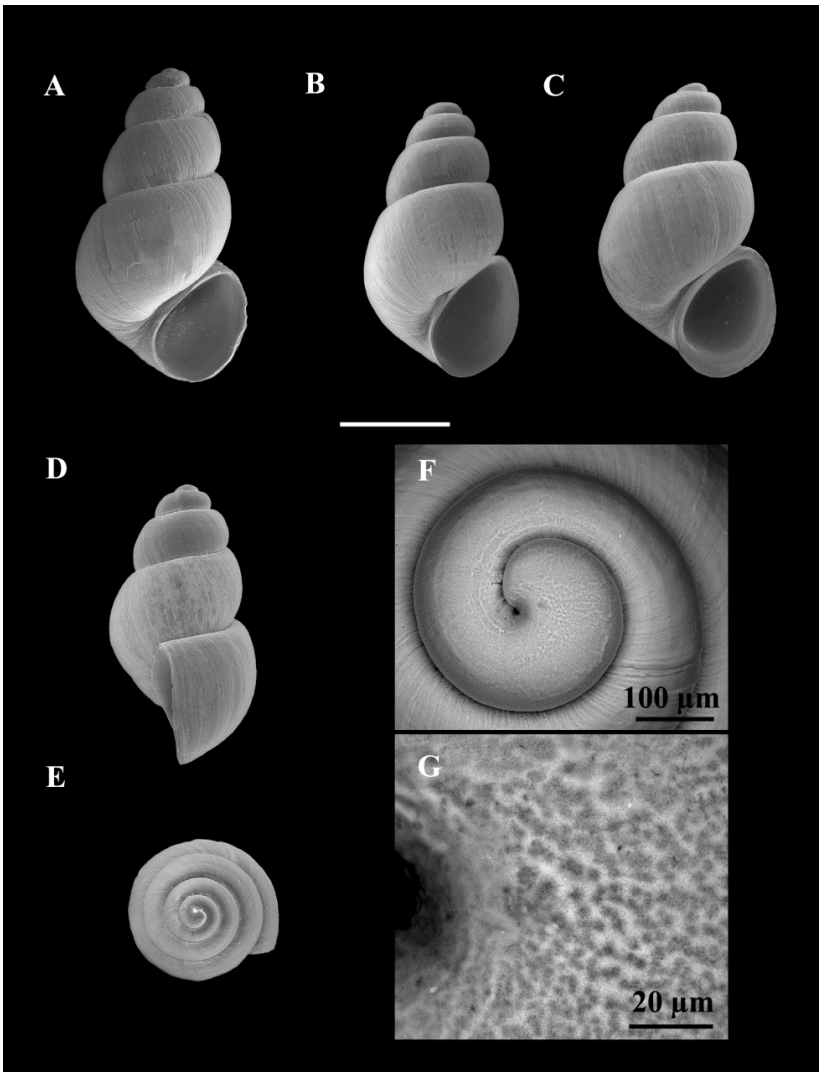


Figure 30. Shells of *Pseudamnicola (P.) gasulli*. **A.** Shell from a stream at Barranco de las Negras, Almería, Spain. **B.** Shell from Retamar Rambla, Almería, Spain. **C-G.** Shells from a stream at Rambla de los Yesos, Alboloduy, Spain. **E-G.** Protoconch and microsculpture of the protoconch.

Description

Shell ovate-conic (Figures 30A-C), yellowish periostracum with 4-5.5 spire whorls, and a height of between 2.0-4.0 mm (Appendix III: Table 1); protoconch approximately 375 µm wide with 1.5 whorls and a nucleus around 100 µm long (Figures 30E, F); protoconch microsculpture granulated (Figure 30G); body whorl about two-thirds the total length; whorls convex with deep suture; peristome frontal, complete, oval, with thick inner lip partially hiding umbilicus; outer peristome simple, straight (Figure 30D).

Operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral, with nucleus submarginal (Figures 31A, B; Appendix III: Table 2); oval muscle attachment near nucleus.

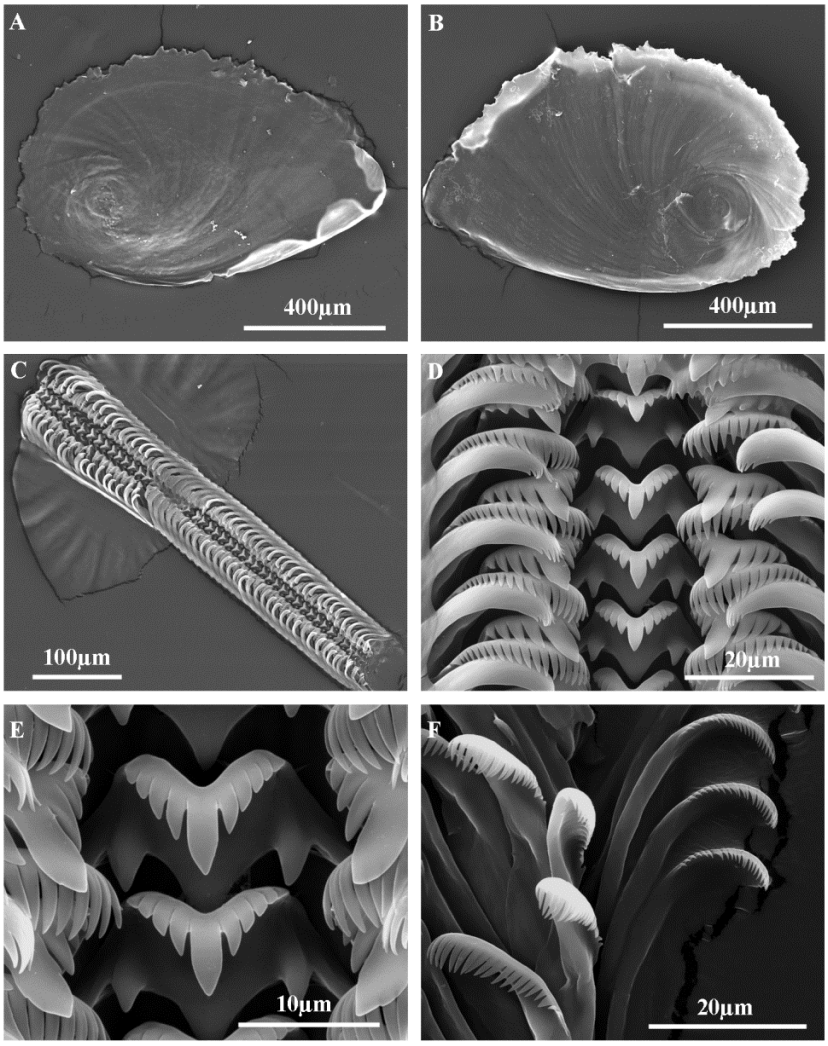


Figure 31. Operculum and radula of *Pseudamnicola (P.) gasulli* from Retamar Rambla, Almería (operculum) and stream at Barranco de las Negras, Almería (radula). **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central teeth. **F.** Outer marginal teeth.

Radula medium in size (15% total shell length) with around 50 rows of teeth, (Figure 31C, Appendix III: Table 3); central tooth with a pointed median cusp and four or five pointed lateral cusps (Figures 31D, E); lateral teeth with a long tongue-

shaped median cusp and four tapered lateral cusps; inner and outer marginal teeth bear approximately 20 and 22 sharp cusps, respectively (Figures 31D, F).

Pigmentation and anatomy: Head intensely brown pigmented from snout to neck except around ocular lobes (Figure 32F); pigment on neck less pigmented than on head; tentacles pigmented except on along a longitudinal stripe; snout as long as wide, with medial lobation; foot of intermediate length, pigmented on dorsal region. Ctenidium in the anterior region of pallial cavity with about 18-23 gill filaments; osphradium ellipsoidal under central gill filaments (Figure 32C, Appendix III: Table 4). Stomach slightly longer than wide (Appendix III: Table 4); style sac slightly shorter than stomach, surrounded by the intestine slightly pigmented in its proximal part (Figure 32E).

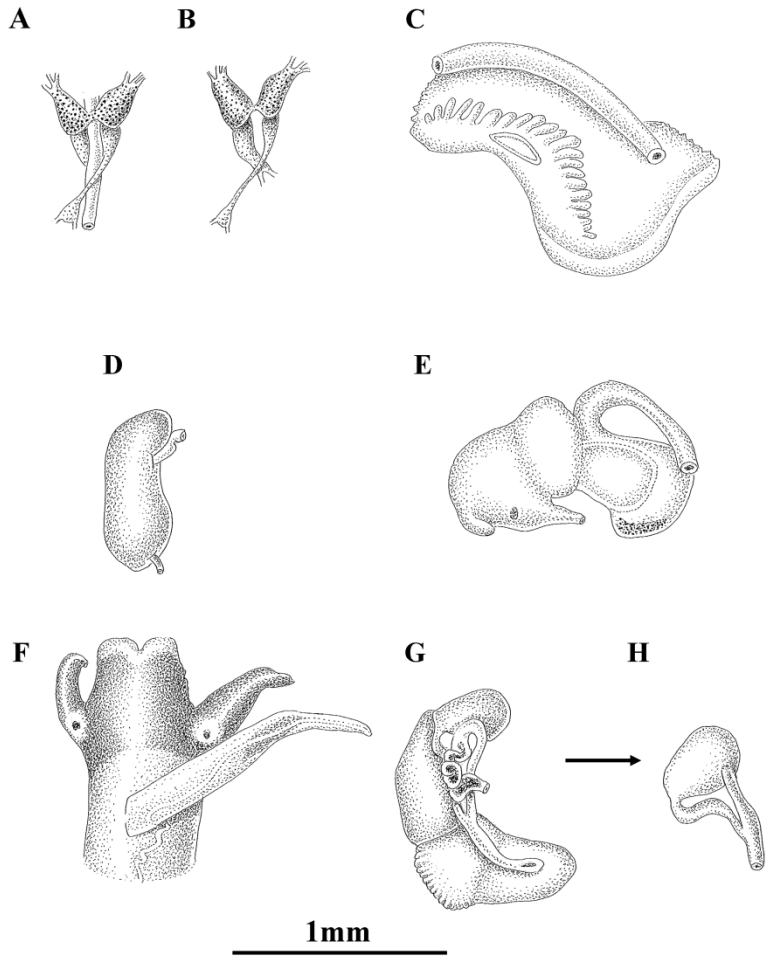


Figure 32. Anatomy of *Pseudamnicola (P.) gasulli* from a stream at Barranco de las Negras, Almería, Spain. **A, B**. Partial nervous system. **C.** Ctenidium and osphradium. **D.** Prostate gland. **E.** Stomach. **F.** Head and penis of a male. **G.** Female genitalia. **H.** Bursa copulatrix.

Female genitalia with a pallial oviduct about three times longer than wide (Figure 32G, Appendix III: Table 5); capsule gland and albumen gland similar in size; the capsule gland is thicker in posterior region; pyriform bursa copulatrix with a duct approximately one and a half times longer than bursa length; in some specimens, bursal duct has a thickening of the duct in its mid-section; renal oviduct black pigmented until loop and very coiled; absence of seminal receptacle (Figure 32H).

Male genitalia bear a bean-like prostate gland about two times longer than wide (Figure 32D, Appendix III: Table 6); strap-like penis with black pigment in the middle region and a narrow base attached to central area of head (Figure 32F); coiled penis observed in some living specimens; uncoiled vas deferens running straight, close to the external margin.

Nervous system brown pigmented, cerebral ganglia equal in size and darker than other ganglia, connectives and commissures; supraoesophageal ganglion larger than suboesophageal; supraoesophageal connective around five times longer than suboesophageal (Figures 32A, B; Appendix III: Table 7). Mean RPG ratio 0.50 (elongate).

Remarks. In 1981, Boeters described this species from the island of Ibiza. It was also later discovered in the provinces of Almería (Boeters, 1988) and Murcia (Suárez y Vidal-Abarca, 1983). After visiting all of these regions, we only found the species in some localities from Almería province; hence, we can only compare anatomical features of the collected specimens from Almería with Boeters' illustrations. The intraspecific variability is mainly reflected in the sizes of the shell, penis and bursa copulatrix. Individuals from these localities share characteristics originally described for this species (Boeters, 1981), that is, an ovate-conic shell, a pyriform bursa copulatrix, the absence of a seminal receptacle and a strap-like penis with black pigment in the middle region.

These characteristics, especially the absence of a seminal receptacle and the presence of a strap-like penis, make *P. (P.) gasulli* exceptional within the genus as all other *Pseudamnicola* species share these traits. Another important difference in character state is the size of prostate gland: *P. (P.) gasulli* has the smallest prostate gland of any of the species within the genus. Genetically, this species is distant from species of both *Pseudamnicola* subgenera. For instance, *P. (P.) gasulli* differs from *P. (Corrosella)* spp. by 11.1% for COI, 8.0% for 16S and 5.6% for 28S and by 12% for COI, 6.4% for 16S and 6.4% for 28S from the other ibero-balearic *P. (Pseudamnicola)* spp.

Material for molecular analyses

Populations of *Pseudamnicola (Pseudamnicola)* from 20 localities in the Iberian Peninsula and Balearic Islands were examined genetically (see Table 9).

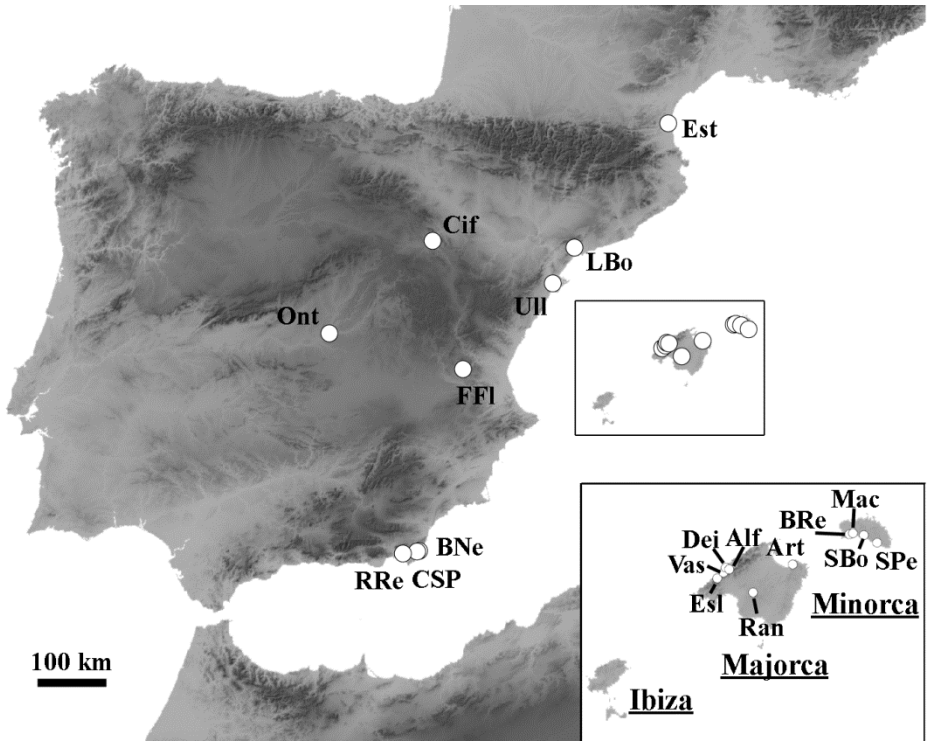


Figure 33. Distribution map of *Pseudamnicola (Pseudamnicola)* populations in the Ibero-Balearic region that were molecularly examined. Codes of localities are displayed in Table 9.

A total of 72 specimens were analyzed in this study, comprising 67 ingroup individuals (Table 9), three specimens of *P. (Corrosella) falkneri* (type species of the other subgenus) and one for the species *Peringia ulvae* and *Mercuria emiliana* as outgroups.

Table 9. Coding and locality name for species sequenced in this study.

Pseudamnicola (Pseudamnicola) species	Locality	Code
artanensis	Spring near Betlem Hermitage, Artá, Majorca Island, Spain	Art
beckmanni	El Rentador Spring, Deià, Majorca Island, Spain	Dei
	Spring in Valldemossa, Majorca Island, Spain	Vas
	Alfabia Gardens, Majorca Island, Spain	Alf
	Stream at the gardens of the Palace La Granja, Esporles, Majorca Island, Spain	EsA
	Spring in Randa, Majorca Island, Spain	Ran
gasulli	Stream at Barranco de las Negras, Almería, Spain	BNe
	Spring at Cala de San Pedro, Almería, Spain	CSP
	Retamar Rambla, Almería, Spain	RRe
granjaensis	Fountain near the exit of the Palace La Granja, Esporles, Majorca Island, Spain	EsB
meloussensis	Stream at Macarella Creek, Minorca Island, Spain (type locality)	Mac
	Font de la Reina, Son Saura, Minorca Island, Spain	BRe
	Stream at Barranco de Son Boter, Minorca Island, Spain	SBo
	Stream at Barranco de Sen Penyes, Minorca Island, Spain	SPe
subproducta	Font Estramar near Salses le Château, Southern France	Est
	Les Borges del Camp, Tarragona, Spain	LBo
	Baltasar Ullal, Tarragona, Spain	Ull
	Prado de Cifuentes Spring, Calatayud, Zaragoza, Spain	Cif
	Ontígola Lagoon, Madrid, Spain	Ont
	Flores Spring, Requena, Valencia, Spain	FFl

Phylogenetic analysis and genetic diversity

Mitochondrial data set. A total of 1166 bp was obtained for COI and 16S gene fragments (658 bp for COI and 508 bp for 16S): 839 were invariant, 95 were variant but parsimony-uninformative and 232 were parsimony-informative. Maximum genetic divergences within the ingroup (measured as the mean of uncorrected *p* distances, Appendix IV: Table 1) occurred between *P. (P.) gasulli* and *P. (P.) subproducta* for COI and 16S (12.4% and 7%, respectively) In the MP topologies, for the COI fragment, *P. (C.) falkneri* was poorly supported as the sister group of *P. (P.) gasulli*, was placed basal to the *P. (Pseudamnicola)* spp. clade). In the ML and BI analyses conducted with COI, *P. (P.) gasulli* was grouped as the sister species of *P. (C.) falkneri*, whereas in the 16S topologies, although not highly supported, all the studied species of *P. (Pseudamnicola)* clustered together as a monophyletic group. Furthermore, in both inferences, the relationship between *P. (P.) subproducta* and *P. (P.) meloussensis* is incongruent. The Minorcan specimens of *P. (P.) meloussensis*, Sen Penyes and Font de la Reina, were clustered within the *P. (P.) subproducta* cluster in the 16S analyses, though this was poorly supported. In contrast, analyses of COI recovered monophyletic groups for these two species.

Nuclear data set. Within *P. (Pseudamnicola)* specimens, the nuclear 18S fragment (518 bp) sequences did not show genetic divergences among pairs of taxa. Therefore, a partial 28S gene fragment was sequenced consisting of 1052 bp of which 897 were invariant, 45 were parsimony-uninformative and 110 were parsimony-informative. The range for divergences between *P. (Pseudamnicola)* species was from 0.1% (*P. (P.) beckmanni* vs *P. (P.) granjaensis*) to 6.4% (*P. (P.) gasulli* vs *P. (P.) subproducta*) (Appendix IV: Table 1). The topology of the tree recovered for both the ML and BI analyses grouped the *P. (Pseudamnicola)* spp. as a monophyletic group, divided into two clades: one clade consisting of *P. (P.) gasulli* and the other clade of the species from the islands of Majorca and Minorca and *P. (P.) subproducta* from the Iberian Peninsula. However, within the second clade, the relationships among species were not well resolved.

Combined data set. A total of 2218 bp was examined after combining the COI, 16S and 28S partitions. The recovered BI tree is shown in Figure 34. The partition homogeneity test indicates that the combined matrix was not fully consistent (P = 0.01). However, this test is considered to have a high degree of type I errors when employing data sets with low levels of significant heterogeneity (because of consistent underestimation of character change inherent to the parsimony method). Despite this caveat, we used the test as it provides more support to the branches when the information from the different fragments was combined. Following the jModeltest, the general time reversible model plus invariant sites plus gamma distribution, GTR + I + G, was the selected substitution model that best fits the combined data set (substitution rates A–C=1.00, A–G=6.63, A–T=3.45, C–G=1.59,

C–T=9.75, G–T=1.00; base frequencies A=0.25, C=0.21, G=0.25, T=0.28; I=0.59; α=0.90). Although analyses of the ribosomal genes (16S and 28S) did not cluster every *P. (Pseudamnicola)* spp. described for the Ibero-Balearic region (except for *P. (P.) gasulli*) as monophyletic groups, the combination of these fragments with the COI fragment does group the sequences according to the species, as monophyletic groups, in all inferences (MP, ML and BI). Within this monophyletic grouping, the species seem to cluster into three well-sustained clades. Although in the ML analysis the relationship between *P. (P.) gasulli* and the other two clades was less certain, in both the ML and BI analyses, the species resolve into the following clades: clade I containing *P. (P.) subproducta* and *P. (P.) meloussensis* from the Iberian Peninsula and Minorca Island, respectively; clade II consisting of the species *P. (P.) artanensis*, *P. (P.) beckmanni* and *P. (P.) granjaensis* from Majorca Island; and clade III comprising *P. (P.) gasulli* from populations in the Iberian Peninsula (as no specimens from Ibiza were found).

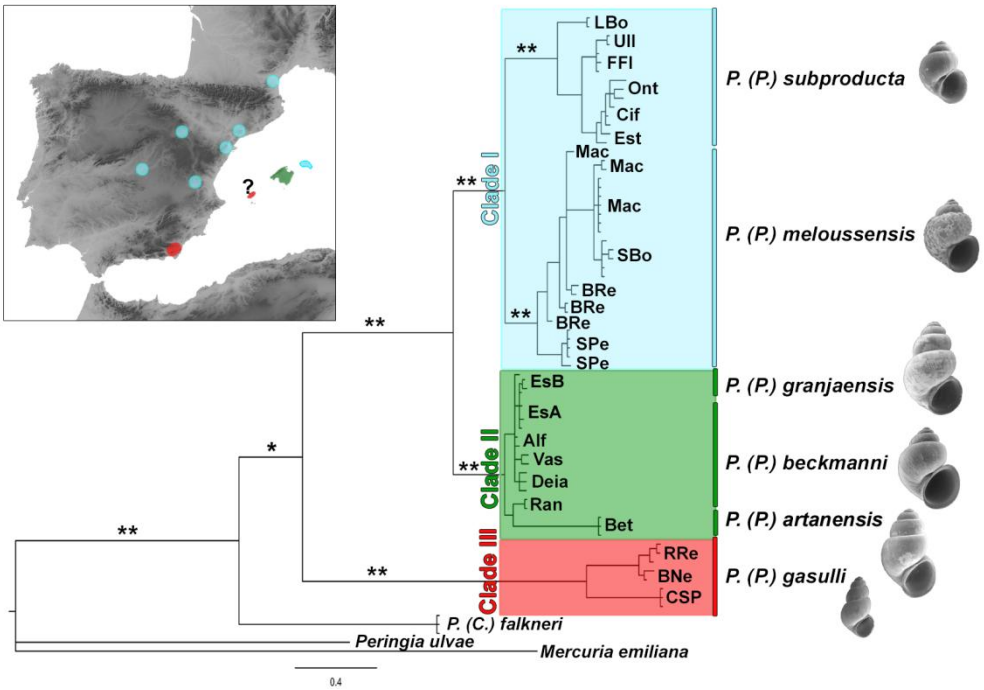


Figure 34. Phylogenetic relationships of the Ibero-Balearic *Pseudamnicola (Pseudamnicola)* species based on the Bayesian topology recovered from the combined data set of mitochondrial (COI and 16S) and nuclear (28S) fragments. The "?" and coloring of Ibiza Island represents the fact that although populations of *P. (P.) gasulli* from Ibiza were not found in our study, it has been previously cited for this location. For locality codes, see Table 9. Supports of branches are given above species level as follows: ** represents support values ≥ 95% and Posterior Probabilities ≥ 0.95 and * indicates support values of MP ≥ 95%, ML between 50% and 75% and Posterior Probabilities ≥ 0.95.

Divergence time estimates

Using coalescence methods and a trait specific rate for COI of 0.81% ± 0.24% sequence substitutions per million years, which is based on rates from other publications (e.g. 0.91% in Wilke, 2003; 0.81% in Hershler and Liu, 2008; a mean of 0.72% in Wilke *et al.*, 2009), the substitution rates of the 16S and 28S fragments were estimated at 0.5 and 0.2 % sequence substitutions per million years, respectively, with very low standard deviations.

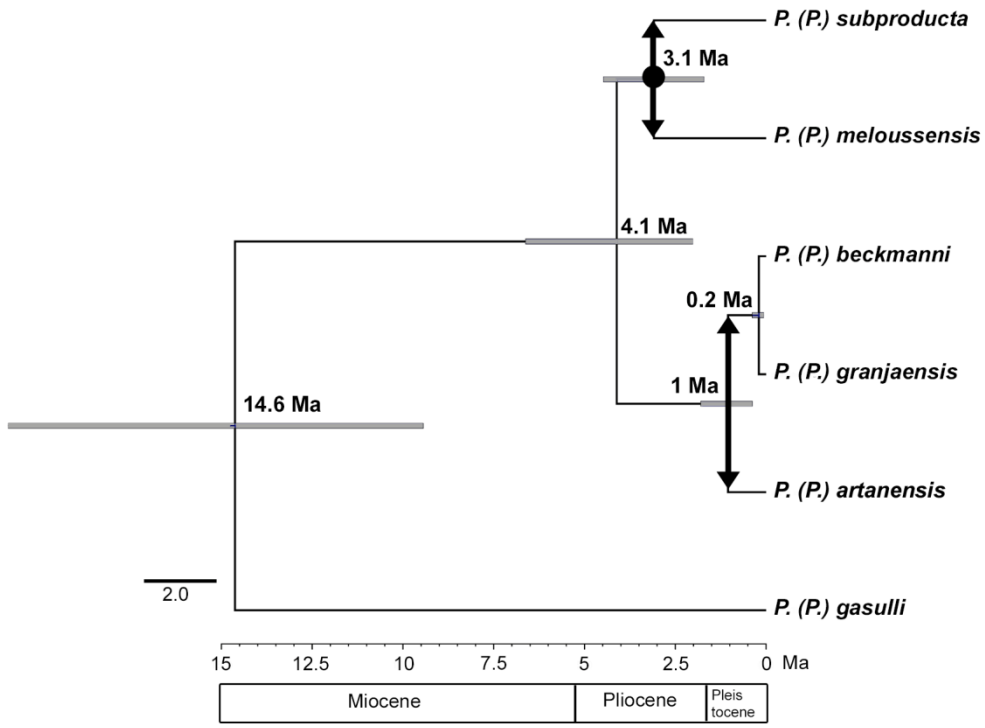


Figure 35. *BEAST chronogram of *Pseudamnicola (Pseudamnicola)* populations from the Ibero-Balearic region based on combined analysis of COI (rate previously calculated) and 16S and 28S ribosomal fragments (rates estimated). *BEAST posterior probabilities were 0.99, except for the one indicated with a black dot, which was 0.75. Grey bars indicate 95% highest posterior density intervals. Black arrows on branches indicate levels of significance using the relative rate test (RRT).

The Relative Ratio Test showed no constancy in rates along the phylogeny (especially in the branches leading to the *P. (P.) meloussensis* and *P. (P.) subproducta* group and the Majorcan species group) and therefore, a strict molecular clock approach is rejected in this study. According to the relaxed-clock model (Figure 35), the earliest split in the *P. (Pseudamnicola)* complex lineage, separating *P. (P.) gasulli* from the rest of the species, occurred during the Miocene (21-9 Ma; mean 14.6 Ma). The subsequent split leading to clades I and II is estimated to have

happened during the late Miocene to Pleistocene between 6.6 Ma and 2 Ma (mean 4.1 Ma). The estimated time range for the divergence between *P. (P.) meloussensis* (Minorca Island) and *P. (P.) subproducta* (Iberian Peninsula) is from 4.5 Ma to 1.7 Ma (mean 3.1 Ma). Lastly, the diversification period of species inhabiting Majorca Island all cluster during the Pleistocene between 1.8 and 0.1 Ma.

Diversification factors

In the absence of outgroups, Mantel tests for the three genes reveal no statistical correlation between genetic divergences and measured parameters, such as water conductivity, altitude and geographic distance (Figure 36, Table 10). The only significant correlation found is between geographic and genetic distances (p values in Table 10), showing a pattern of isolation by distance.

Table 10. Mantel test results for each gene partition comparing the genetic distance matrix (including the uncorrected p-distances) with distance matrices of the abiotic variables of water conductivity, locality altitude and geographic distances between two populations. The value of n represents the number of localities included in each analyzed correlation, *r* is the correlation coefficient and *p* is the statistical significance.

Genetic distances	Independent variable	n	r	p
COI	Conductivity	13	0.06	0.168
	Altitude	17	0.02	0.334
	Geographic distance	17	0.62	0.001
16S	Conductivity	13	0.21	0.05
	Altitude	17	-0.03	0.448
	Geographic distance	17	0.63	0.001
28S	Conductivity	13	-0.10	0.684
	Altitude	17	-0.08	0.656
	Geographic distance	17	0.58	0.001

In addition to the 20 populations examined genetically here, we include data for ten other localities in the Ibero-Balearic region for *P. (Pseudamnicola)* species. After plotting the 30 collecting points on a map and using GIS to extract additional data, such as the age, petrology and pH of soils, no pattern was observed between these variables and *P. (Pseudamnicola)* spp. distribution. Extracting the information given by the layer of the age of soils, we could observe that the geomorphology among the Balearic Islands is very similar, consisting mainly of Mesozoic and

Cenozoic rocks, except for Minorca Island, which is also composed of Paleozoic rock. Regardless, these results indicate that *P. (Pseudamnicola)* species are widespread over the islands without any pattern or preference related to rock type.

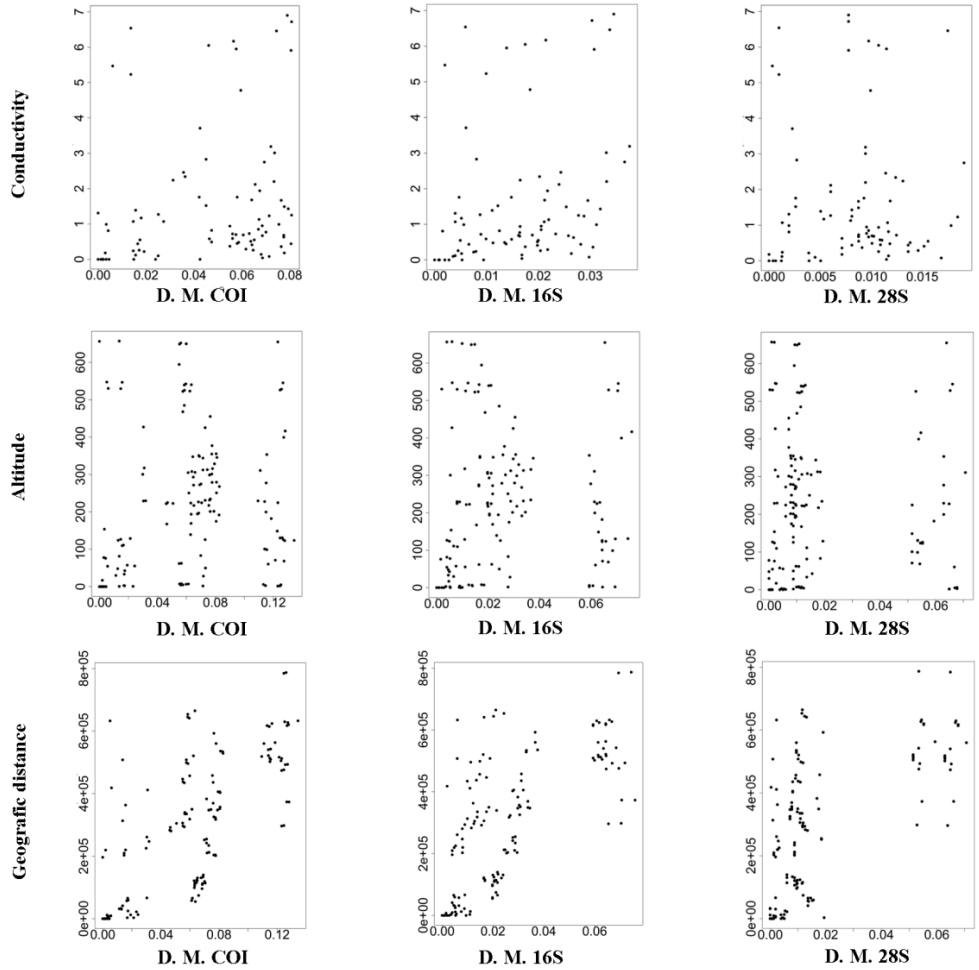
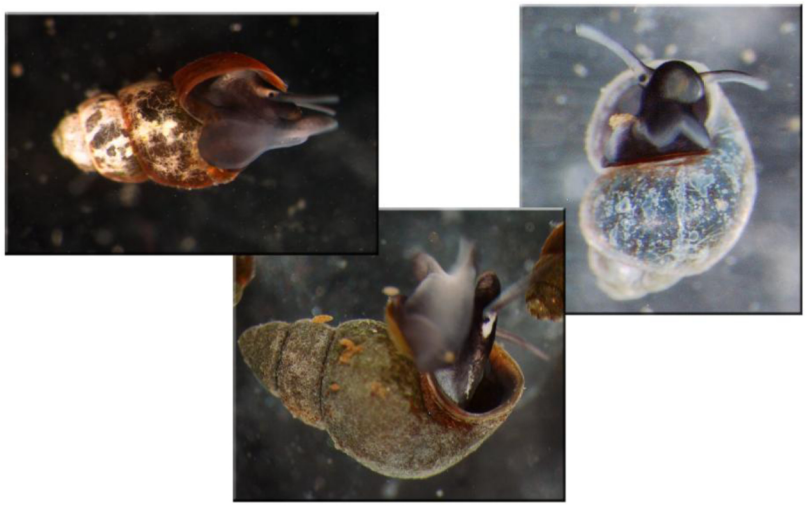


Figure 36. Mantel test scatterplots showing the correlation between the genetic distance matrix (D. M.) of each studied gene and distance matrices of conductivity, altitude and geographic distances.

2. TAXONOMÍA Y FILOGENIA DEL SUBGÉNERO *PSEUDAMNICOLA (CORROSELLA)* EN LA REGIÓN ÍBERO-BALEAR



SUBGÉNERO *PSEUDAMNICOLA (CORROSELLA)* (BOETERS, 1970)

Type species

Pseudamnicola (Corrosella) falkneri (Boeters, 1970)

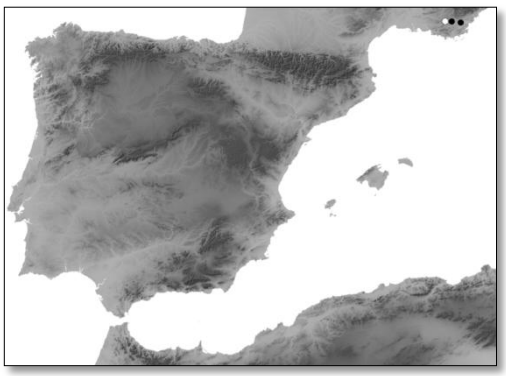
Diagnosis

Shell ovate-conic, generally eroded, and longer than wide; female genitalia with pyriform bursa copulatrix and one short seminal receptacle, either elongated or pyriform; penis gradually tapering with the base expanded and some folds in middle section; dark patch of pigment in penis, with different sizes, from the middle region to the tip; nervous system from moderately concentrated to elongated (RPG ratio).

Composition

Thirteen species, including seven new species, six out of them recently published by the author:

Pseudamnicola (Corrosella) astieri (Dupuy, 1851)



Hydrobia astierii Dupuy, 1851: 556-557, pl. XXVII, fig. 12, Paris (Type loc. Surroundings of Grasse, Alpes-Maritimes, France [shell description]).

Paludinella astieri (Dupuy): Frauenfeld 1865: 575.

Bythinella astieri (Dupuy): Locard 1882: 227; Berenguier, 1882: 83; Locard, 1893: 79, fig. 81 (shell description); Berenguier, 1902: 378, pl. 16 fig. 6 (1990).

Bythinella anteisensis Berenguier, 1882: 83, 89-90 (Type loc. Foux de Draguignant, Var, France [shell description]); Berenguier, 1902: 378-379 (shell description) pl. 16 fig. 7 (1990). (Synonymy: Girardi, 2009: 56).

Bythinella berenguieri Bourguignat in Berenguier, 1882: 83, 99-100 (Type loc. Foux de Draguignant, Var, France [shell]); Berenguier, 1902: 379-380 (shell description) pl. 16 fig. 8 (1990) (Synonymy: Girardi, 2009: 56).

Bythinella doumeti Bourguignat in Locard, 1893: 91. (Type loc. surroundings of Nimes, Gard, France [shell description]) (Synonymy: Falkner *et al.*, 2002: 81, after revision of two syntypes in the Bourguignat collection).

Corrosella anteisensis (Berenguier): Boeters, 1970: 64, figs. 2, 4, 7, 9 [(shell, operculum, male and female genital systems of topotypes; Boeters could not find the syntypes)], (=*Bythinella berenguieri* Bourguignat in Berenguier). (Synonymy: Girardi, 2009: 56).

Pseudamnicola (*Corrosella*) *astierii* (Dupuy): Falkner *et al.*, 2002: 29, 80-81; Girardi 2009: 56-61, figs. 1-3 (Var, France: Source d'Argens, Source du Pavillon, Source de la Foux à Draguignan [shell and anatomy]).

Type locality

Surroundings of Grasse, France (Dupuy, 1851).

Type material

Boeters (1970) reported the existence of one specimen with the label "*Paludinella astieri*, typus ex Dupuy" in Paladilhe's collection at the Faculté des Sciences, Montpellier, France. We tried in vain to confirm the existence of such material at the university mentioned. Consequently, we should consider that the type specimen is presently inaccessible for study. However, some topotypes of *Corrosella anteisensis* (Berenguier) from Foux à Draguignan, Var exist: BOE 261, 285 a-c, 291b Boeters (1970) and Girardi (2009). This author also reported *P. (C.) astieri* from Source d'Argens, Brue-Aurillac à Seillons, Var Department and the Source du Pavillon, Ruisseau Fauvery à Ponteves, Var (Girardi, 2009).

Material examined

A few specimens collected from Source d'Argens, Brue-Aurillac, Var, France after finding the type area and other localities in Alpes-Maritimes and Var practically

destroyed by severe storms. A total of two females and four males have been examined for anatomical descriptions (Appendix III: Tables 1-7).

New diagnosis

Shell yellowish or whitish with a body whorl occupying 2/3 shell length and a deep suture between whorls; protoconch microsculpture granulated; central radular tooth formula 7-C-7; style sac surrounded by a black pigmented intestine; elongate bursa copulatrix U-shaped; elongate seminal receptacle without duct; penis slender with a black patch of pigmentation and some folds in its middle region; nervous system brown pigmented with supraoesophageal connective about two times longer than suboesophageal.

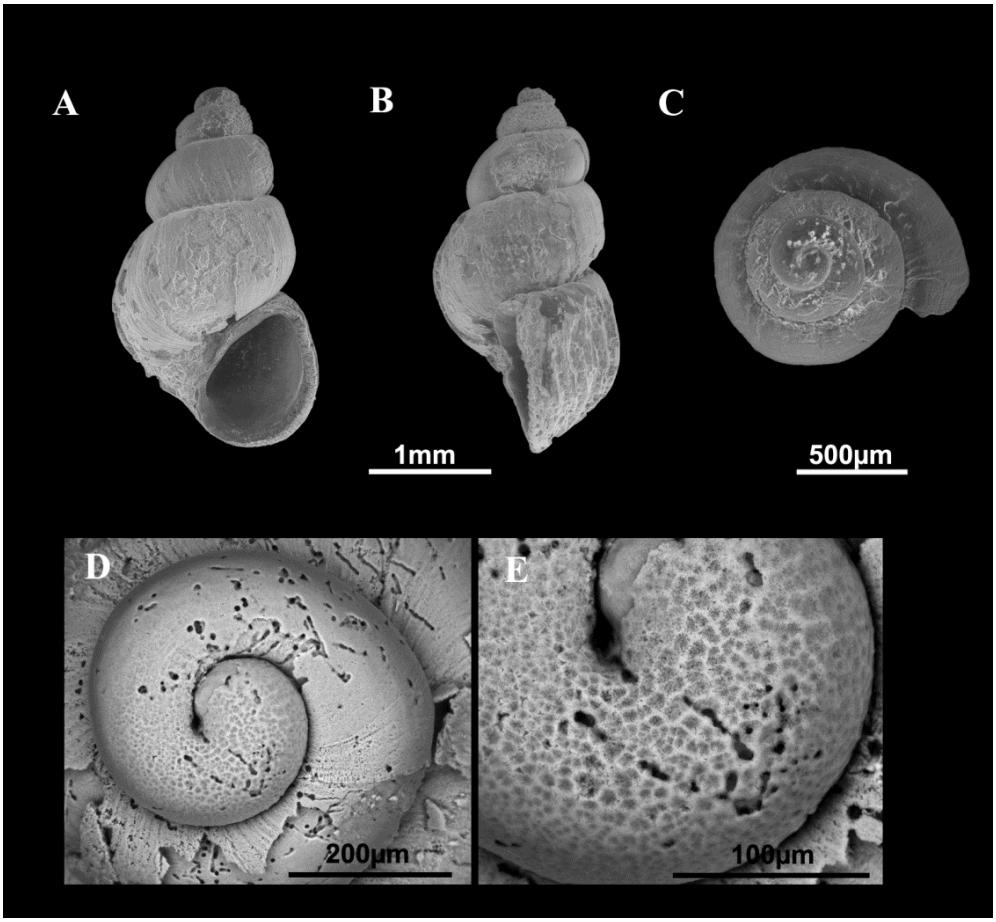


Figure 37. Shells of *Pseudamnicola (C.) astieri*. **A-E.** Shells from Source d'Argens, Brue-Aurillac, Var, France. **D-E.** Protoconch and detail of the microsculpture from the protoconch.

Description

Shell ovate-conic with 4-4.75 spire whorls, height 2.5-3.5 mm (Figures 37A, B, Appendix III: Table 1); periostracum yellowish or whitish; protoconch approximately 370 μm wide with 1.5 whorls and a nucleus around 150 μm long (Figures 37C, D); protoconch microsculpture granulated, more intense on apex (Figure 37E); body whorl about 2/3 total length; teleoconch whorls convex with a deep suture; aperture complete, oval, with an inner lip thicker than outer lip; peristome margin simple, straight (Figure 37B).

Operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral with nucleus submarginal (Figures 38A, B, Appendix III: Table 2); muscle attachment area oval, located near the nucleus.

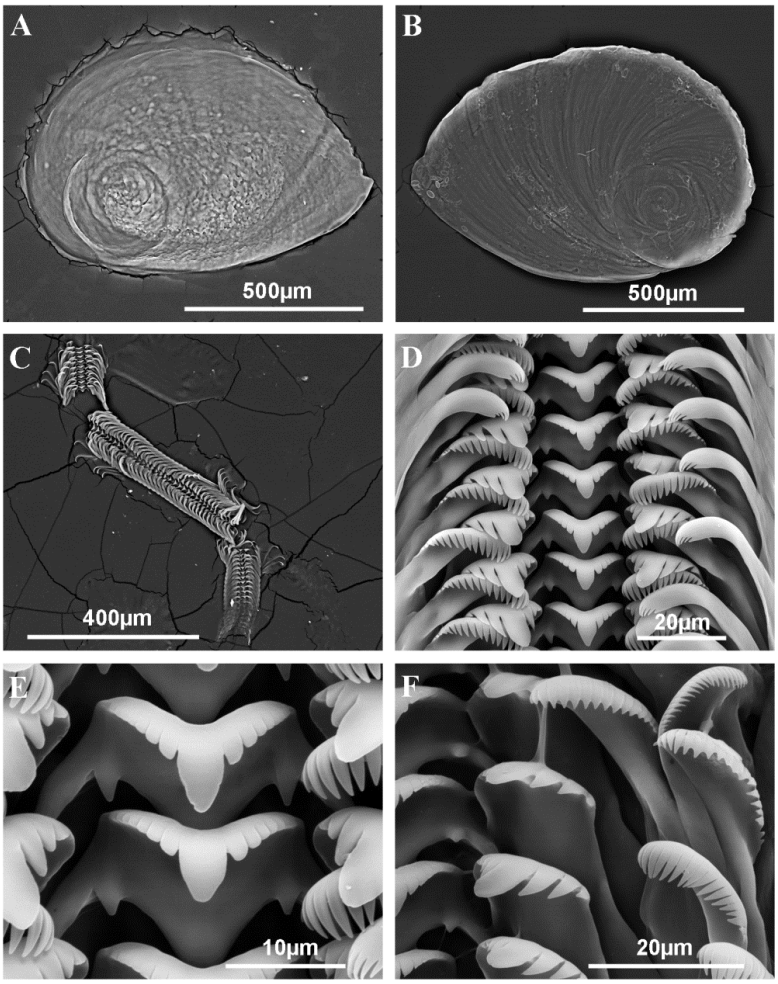


Figure 38. Operculum and radula of *Pseudamnicola (C.) astieri* from Source d'Argens, Brue-Aurillac, Var, France. **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central tooth. **F.** Lateral, internal and external marginal teeth.

Radula of intermediate length (20% total shell length), bearing some 50 rows of teeth (Figure 38C, Appendix III: Tables 3); central tooth has a tongue-shaped median cusp and seven blunt lateral cusps (Figures 38D, E); lateral teeth with three tapered cusps on each side of a long central tongue-shaped cusp; inner marginal teeth have 18 sharp cusps, shortening towards the tooth base; outer marginal teeth with 19 sharp cusps (Figures 38D, F).

Pigmentation and anatomy: Head dark brown pigmented from snout to neck (Figure 39D); pigmentation clearer on neck; tentacles also brown pigmented except for a narrow band on these and on ocular lobes; snout long as wide, with medial lobation; foot of intermediate length and pigmented in dorsal region. Ctenidium in middle region of pallial cavity filling ca. 70% of its length with 17-18 gill filaments; osphradium of intermediate width under central gill filaments (Figure 39C, Appendix III: Table 4). Stomach slightly longer than wide with a small posterior caecum; style sac shorter than stomach and surrounded by intestine black pigmented (Figure 39F, Appendix III: Table 4).

Female genitalia with a slender pallial oviduct (Figure 39G; Appendix III: Table 5); capsule gland longer than albumen gland and consisting of two regions, the posterior one being more transparent; elongate bursa copulatrix, long, folded and U-shaped with a duct about 70% of bursa length; renal oviduct straight and less pigmented from the insertion point of the bursal duct to where it begins to fold and black pigmented making one or two loops; elongate seminal receptacle without duct (Figure 39H) joining renal oviduct just before the point where the bursal duct joins the renal oviduct.

Male genitalia bear a bean-shaped prostate gland about three times longer than wide (Figure 39E, Appendix III: Table 6); penis long, slender, with a black patch of pigmentation and some folds in its middle region; attachment area behind right eye (Figure 39D); penial duct scarcely visible running straight close to the outer penis margin.

Nervous system brown pigmented, consisting of disperse points of pigmentation; cerebral ganglia equal in size; supraoesophageal connective more than two times longer than suboesophageal (Figures 39A, B; Appendix III: Table 7). Mean RPG ratio 0.42 (moderately concentrated).

Remarks. The only available information on the anatomy of this species in the literature corresponded to populations from Foux a Draguignan (figures 2, 4, 7, 9 in Boeters, 1970 and figure 2 by Bodon in Girardi, 2009) and Source du Fauvery in Ponteves (figure 1 by Bodon in Girardi, 2009). The specimens examined from Source d'Argens (Brue-Aurillac) are similar in shell and gastric complex shapes to specimens from Source du Fauvery though they more resemble specimens from the Foux à Draguignan in terms of pallial oviduct shape and number of gill filaments.

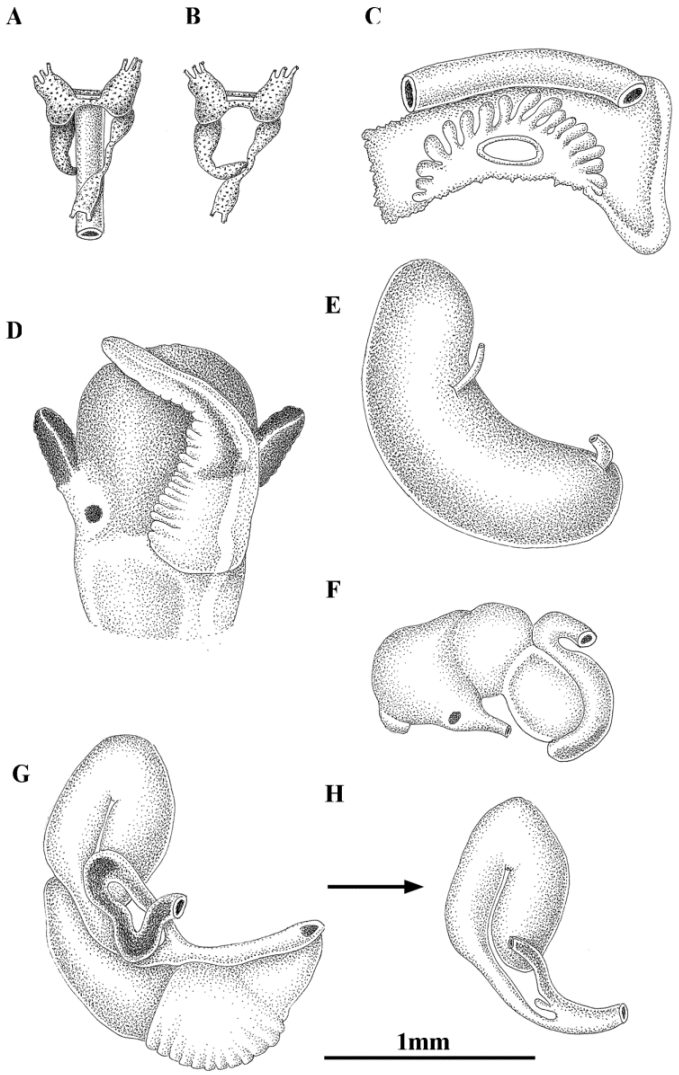


Figure 39. Anatomy of *Pseudamnicola (C.) astieri* from Source d'Argens, Brue-Aurillac, Var, France. **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Head of a male and penis. **E.** Prostate gland. **F.** Stomach. **G.** Female genitalia. **H.** Bursa copulatrix and seminal receptacle.

However, other important diagnostic characters such as the shape of the penis and bursa copulatrix as well as seminal receptacle shape and its position on the renal oviduct are similar in the three populations. Based on these comparisons we conclude that specimens of the three localities belong to the same taxonomic unit with some inter-population variability shown.

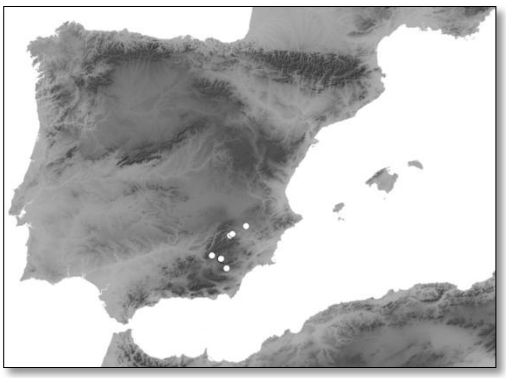
Comparing shell sizes among the *Pseudamnicola (Corrosella)* species from the northern half of Iberian Peninsula, the shells of *P. (C.) astieri* are larger

(2.5-3.5 mm) than those of *P. (C.) hauffei* (2.20-2.90 mm) (see Appendix III: Table 1) and *P. (C.) hinzi* (2.2-2.7 mm, Boeters 1986) yet similar in size to those of *P. (C.) navasiana* (Fagot, 1907) (3.0-3.5 mm, Boeters 1988). The only two shell variables resulting similar between *P. (C.) astieri* and *P. (C.) hauffei* were the rate SL/SW and NSW. That means that both species share the same ovate-conic shape and around 4 spire whorls, which are common characteristics among all *Pseudamnicola (Corrosella)* species. Anatomically, *P. (C.) astieri* bears a similar or higher number of gill filaments (about 17-18) than *P. (C.) hinzi* (16-17, Boeters 1986), *P. (C.) navasiana* (15-16, Boeters 1988) and *P. (C.) hauffei* (about 15). The penis in *P. (C.) astieri* is narrower and more slender than in *P. (C.) hauffei* and *P. (C.) navasiana*, although it is wider and longer than in *P. (C.) hinzi*. The copulatory organ is pigmented in its distal region in all four species, but the pigmentation patch is larger in *P. (C.) astieri*. Although the bursa copulatrix is usually elongate or pyriform shaped among *Corrosella* species, it is U-shaped and folded in *P. (C.) astieri* whereas it is J-shaped in *P. (C.) hauffei*, *P. (C.) hinzi* and *P. (C.) navasiana*. A small seminal receptacle (around 0.15 mm) is a character common to all four species.

Pseudamnicola (Corrosella) falkneri (Boeters, 1970)

Corrosella falkneri Boeters, 1970: p.65-66, figs 1, 3, 5, 8, 10 and 11.

Pseudamnicola (Corrosella) falkneri (Boeters, 1970): Boeters, 1988: 202-203, figs. 66, 70, 89-91, pl. 2 fig. 19.



Type locality

According to its original description this species was collected by Falkner on 28 September 1967 in two streams, separated by 120 m, at "Cerro de la Virgen", Granada, Spain. The eastern flow, between the source and a watering place, is the type locality. The Cerro de la Virgen is located between Galera and Orce above the southern edge of the river Orce (Boeters, 1970). In 1988, Boeters added UTMWG 47 to this type locality.

Type material

Holotype SMF, Paratypes SMF, MP, RNHL, F, BOE 222 et 223 (Boeters, 1970). [MP (=MNHN), RNHL (=RMNH), F (=FALK or Falkner, according to original writing in collection' labels)]. Types: Holotype SMF 219026, Paratypes SMF 219027/1, 219028/10, MNHN, FALK, RMNH, BOE 222 and 223 (Boeters, 1988).

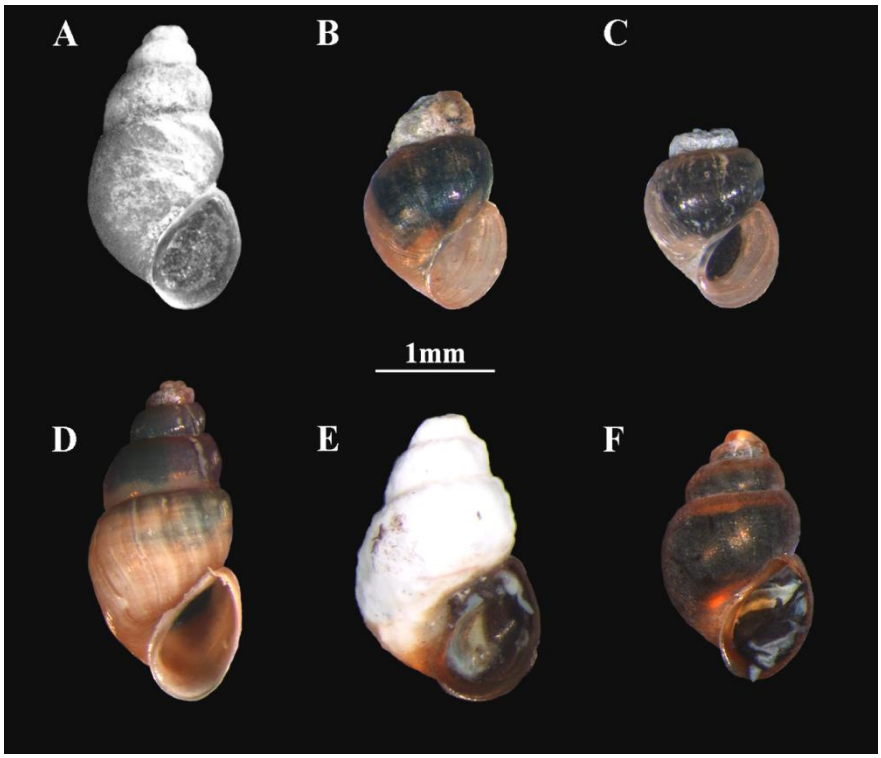


Figure 40. Shells from several populations of *Pseudamnicola (C.) falkneri*. **A.** Holotype (SMF 219026). **B**, **C.** Paratypes (SMF 219027/1, 219028/10). **D.** Shell from La Armada spring, Orce, Granada. **E.** Shell from Dos Caños spring, Castril, Granada. **F.** Shell from Fuente Palo, Orce, Granada.

Material examined

Type material. We examined a photograph of the holotype (SMF 219026) labeled as "Abfluss des östlichen Quellaustritts, Cerro de la Virgen, Granada. Falkner leg. 28/9/67 ex. BOE 222", five shells from SMF 219028/10 labeled as "Paratypen, Spanien: Granada: Cerro de la Virgen: W Quellenaustritt, G. Falkner 28.9.67 (BOE 223b)", 10 shells from the NHMW 107332 labeled as "Paratypen ex BOE 223b. *Corrosella falkneri* Quellenaustritt der W. Balsa, Cerro de la Virgen, Granada FALKNER leg 28 IX 1967", four shells (dry specimens) NHMW 107331 labeled as "Paratypen ex BOE 223b *Pseudamnicola (Corrosella) falkneri* Westl.

Quellenaustritt am Cerro de la Virgen zw. Galera u. Orce oberh. des Süduffers der Vega d. Rio Orce, Granada leg. Falkner 28.9.1967", 15 shells (most of them juveniles) recently collected at 223b (D.M., N.M. and E.M., 20 March 2011, MNCN 15.05/49430).

All published papers (Boeters, 1970, 1988) mentioned the existence of more than one sample collected at locality 223. The presence of material labeled as "BOE 223b" in SMF and as "ex BOE 223b" in NHMW alerted us to the existence of a sample 223a. Boeters (personal communication) kindly clarified that the description of *P. (C.) falkneri* was based on specimens of three samples received from Gerhard Falkner as follows:

- BOE 222 is the type locality ("écoulement oriental", according to Falkner "Abfluss des östlichen Quellaustritts" [flow from the eastern spring emergence]). Type material from this sample: holotype: SMF 219026, paratypes: SMF 219027/1 in addition to BOE collection (Boeters personal communication) that comprises BOE 222 specimens either in alcohol (now BOE 0222) or shells (now BOE 2629).

- BOE 223 is the second original locality ("écoulement occidentale"). However Falkner distinguished between "Acequia unterhalb der westlichen Balsa" [acequia downhill of the western balsa] (BOE 223a) and "Quellaustritt" [spring emergence] (BOE 223b). Paratypes at present in BOE collection (Boeters personal communication) comprise animals in alcohol (from BOE 223a, now BOE 0223) and shells (from BOE 223b, now BOE 2630).

Neither the papers by Boeters (1970, 1988) nor the labels of the collection materials mention the names of the springs, and it was therefore difficult to identify the precise location of the type locality. Boeters (personal communication) provided additional data that have allowed us to locate them. The exact location of the Boeters' samples is: (European Datum 1950): BOE 222, UTM: 30S 543001 4175819, BOE 223a, UTM: 30S 542838 4175797, BOE 223b, UTM: 30S 542840 4175779 (all explored by D.M., N.M. and E.M. 20 March 2011). Water flows near Cerro de la Virgen are now dry or destroyed and only a few empty shells were found at locality 223b (Moreno, personal communication). The area was extensively explored and *P. (C.) falkneri* was found in five springs at the town of Orce close to Orce River. Localities from this town and others found for this species are gathered in Appendix 1.

Material examined for morphometry

Measurements of the holotype based on published photographs (Boeters, 1970, fig. 10; Boeters, 1999, fig. 3) (Figure 40A) and the available paratypes (Figure 40B, C)

were compared with measurements of specimens of all the new localities where the species has been found. As explained below, La Armada spring specimens (Orce) were selected for morphometric comparisons with the other species here examined because they were the least eroded and most had a preserved apex. Shell, anatomical, operculum and radular measurements for La Armada (Appendix III: Tables 1-7) correspond to males and females collected in May and October.

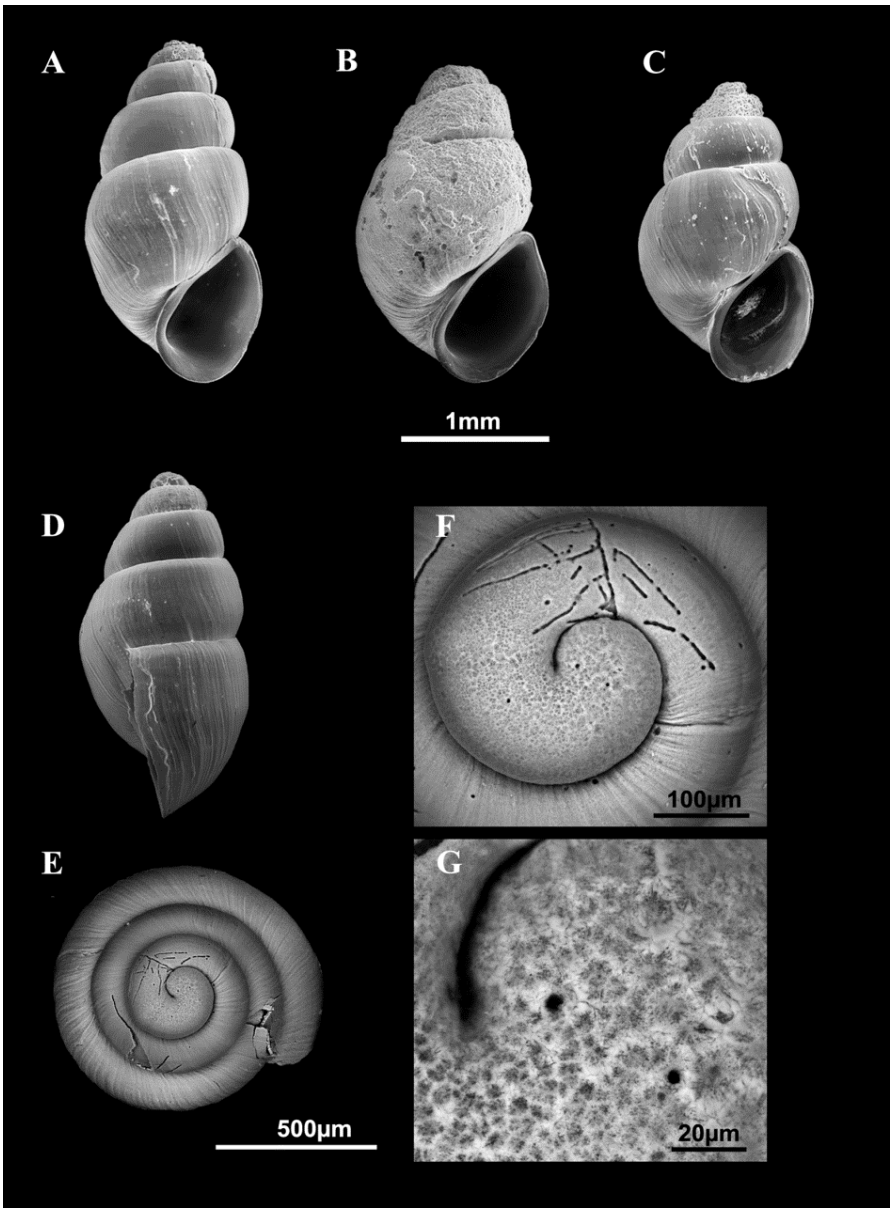


Figure 41. Shells of *Pseudamnicola (C.) falkneri*. **A**, **D-G.** Shells from La Armada spring, Orce, Granada. **B.** Shell from Dos Caños spring, Castril, Granada. **C.** Shell from La Errá spring, La Dehesa, Albacete. **E-G.** Protoconch and microsculpture.

Diagnosis

Shell small with yellowish periostracum; radial sculpture on teleoconch; outer peristome with a slight sinuosity in lateral view; plain central radular tooth weakly serrated and lateral teeth with six lateral cusps at each side of median cusp; bursa copulatrix long cylindrical; seminal receptacle elongated; renal oviduct black pigmented until loop; prostate gland three or four times longer than wide; penis slender tubular with a small black patch of pigment in distal region; nervous system black pigmented with a supraoesophageal connective eight times longer than suboesophageal.

Description

Shell ovate-conic, yellowish periostracum with 4.5-5 spire whorls, height 2-2.6 mm in non-eroded specimens (Figures 40D, 41A-C, Appendix III: Table 1). In some populations, shells are very eroded at their apex or apex is even lost in different measure showing white color in this zone; protoconch with around 1.25 whorls, 350 μm total length and nucleus width approximately 100 μm (Figures 41E, F); granular microsculpture with granules more strongly patched at the protoconch nucleus (Figure 41G); teleoconch with radial sculpture marks on its surface, more marked on body whorl which occupies more than half of the total shell length; peristome oval, complete, with a thin outer lip and a thicker inner lip which is in contact with body whorl hiding the umbilicus; outer peristome simple showing slight sinuosity in lateral view (Figure 41D).

Operculum with around 2.5 spire whorls on internal side; oval muscular attachment mark appears near the nucleus (Figures 42A, B, Appendix III: Table 2).

Radula approximately eight times longer than wide (Figure 42C, Appendix III: Table 3) of intermediate size (representing 30% of total shell length); contains around 62 rows of teeth; central tooth flattened, without cusps, border only weakly serrate (Figures 42D, E) with small basal cusp on each side; lateral teeth contain tongue-shaped central cusps and six laterals (external ones longer than internal); inner marginal teeth have 33 cusps which are smaller towards the tooth base, but the fourth cusp from the tooth base is wider and longer than those surrounding it; outer marginal teeth with 25 cusps occupying over 25% of tooth surface (Figures 42D, F).

Pigmentation and anatomy. Head and tentacles dark brown, but pigment absent from ocular lobes (Figure 43E); foot intermediate in size with dark brown pigmentation on its dorsal side. Ctenidium in the middle of the pallial cavity; contains 12-13 gill filaments taller than long; osphradium opposite middle section of ctenidium (Figure 43C, Appendix III: Table 4). Stomach and style sac longer than wide (Appendix III: Table 4); its anterior region being surrounded by intestine,

pigmented in some specimens (Figure 43F); rectum slightly S-shaped in pallial cavity.

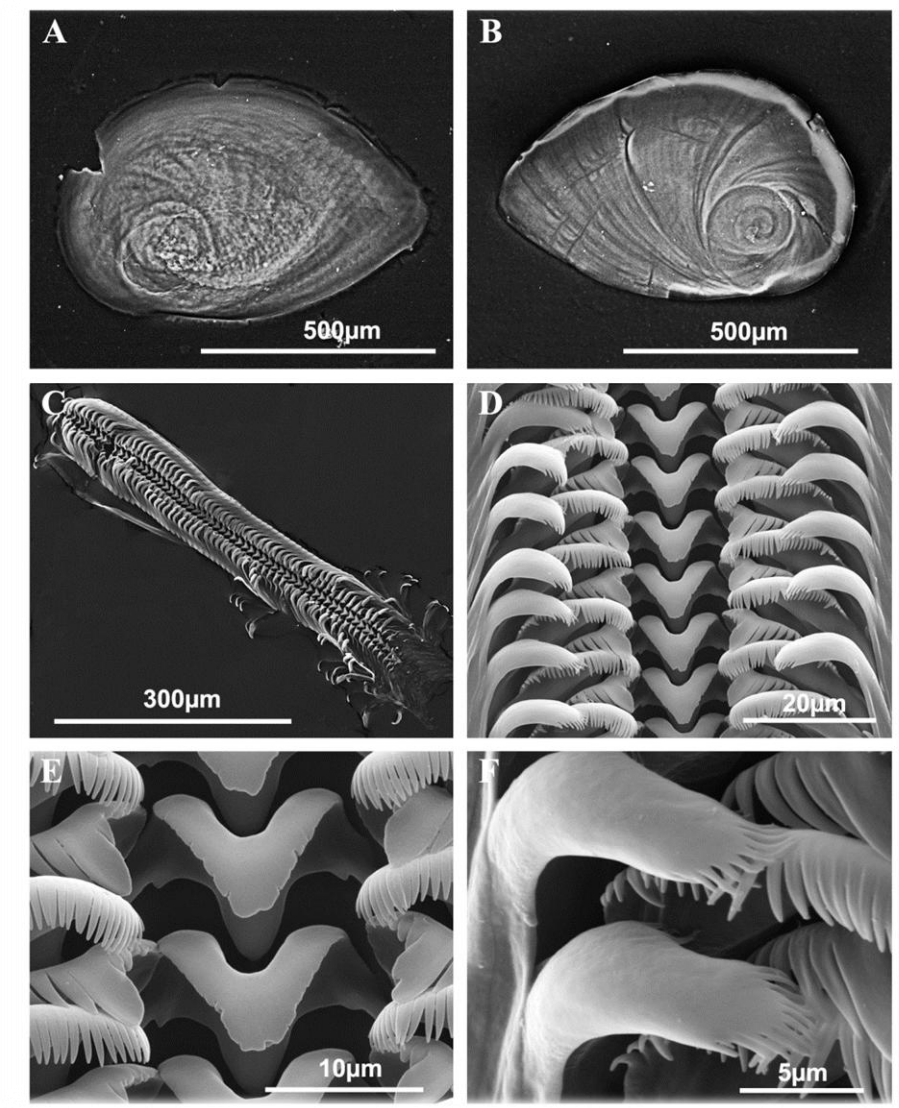


Figure 42. Operculum and radula of *Pseudamnicola (C.) falkneri* from La Armada spring, Orce, Granada. **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central tooth. **F.** Outer marginal teeth.

Two morphologies of female genitalia observed within the same population: one with both glands in the pallial oviduct approximately equal in size (Appendix III: Table 5) and a long cylindrical bursa folded into a J-shape (Figures 43G, H), sometimes its distal end also folds; and the other with a capsule gland longer than albumen gland and cylindrical U-shaped bursa copulatrix (Figures 43I,

J); in both, the renal oviduct is straight and lacks pigment from its joining point with the bursal duct to oviduct loop, thereafter it becomes blackish and undulates; small elongated seminal receptacle without duct lying on renal oviduct above insertion of the bursa copulatrix duct.

Male genitalia with a three or four times longer than wide prostate gland (Appendix III: Table 6), and efferent duct entering into the ventral middle-posterior section and deferent duct emerging in the anterior section (Figure 43D); penis intermediate size with base wider than distal region, flattened with a black small patch of pigmentation in distal region (Figure 43E); straight penial duct runs along the external side of penis.

Nervous system black pigmented, with darker pigment on ganglia; cerebral ganglion equal in size approximately joined by 0.11 mm of commissure; supraoesophageal connective is eight times longer than suboesophageal, which usually consists of a simple strangulation; left pleural ganglion usually larger than right (Figure 43A, B, Appendix III: Table 7). Mean RPG ratio 0.49 (moderately concentrated).

Remarks. After examining the available type material, the labels and the original description (Boeters, 1970 and personal communication 2010) one can conclude that Boeters considered that: (1) all the material collected by Falkner between Galera and Orce (three samples BOE 222, 223a and 223b) was type material, (2) holotype SMF 219026 and paratypes SMF 219027/1 (ex. BOE 222) plus BOE 0222 and BOE 2629 correspond to the type locality "écoulement oriental", (3) the other two samples (223a and 223b) are from the second original locality "écoulement occidentale", (4) specimens SMF 219028/10 and the four specimens NHMW107331 are subsamples of the same sample (ex. BOE 223b) ("Westl. Quellenaustritt"), whereas the provenance of the 10 NHMW 107332 paratypes is still doubtful because of the contradictory information in their original labels; that is, the location "Quellenaustritt derW. Balsa" seems to correspond to the third sample 223a, although the writing on the label reads "ex. BOE 223b".

Figure 40 shows the apex erosion that can be observed in both the paratypes and shells of recently collected specimens. Erosion frequently produces loss of first whorls and therefore most length measurements are useless, the remaining variables being too few for a comparative statistical analysis. The population from La Armada is the most similar to the holotype and accordingly it was used for conchological comparisons with the other species in this study (see Statistical analysis).

Populations from Granada exhibit two morphologies of female genitalia within the same population, though genetic studies (own unpublished data) indicate no divergence between the two morphotypes that would support two different taxonomic entities (see Discussion). However, all females from Albacete have a

larger bursa copulatrix and smaller pallial oviduct, the bursa copulatrix being even longer in populations from Albacete than Granada. These morphological inter-

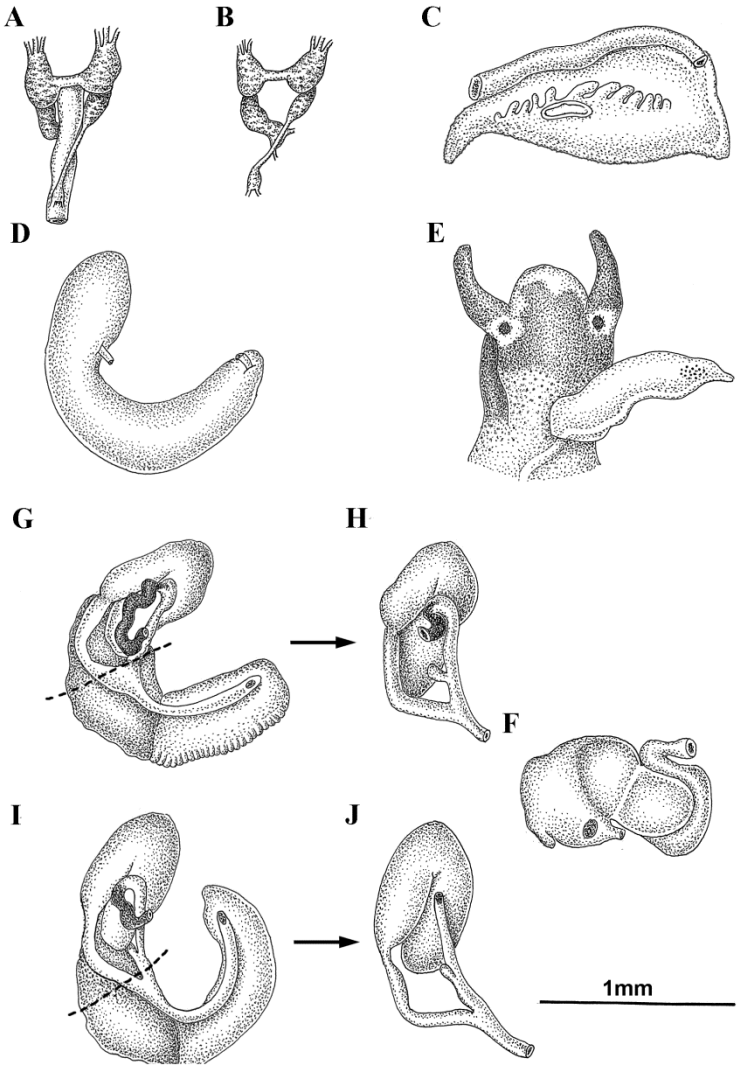


Figure 43. Anatomy of *Pseudamnicola (C.) falkneri* from La Armada spring, Orce, Granada. **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Prostate gland. **E.** Head of a male and penis. **F.** Stomach. **G.** First morphology of female genitalia. **H.** Bursa copulatrix and seminal receptacle of first morphology. **I.** Second morphology of female genitalia. **J.** Bursa copulatrix and seminal receptacle of second morphology. Dotted lines on female genitalia indicate the pallial wall.

population differences are reflected in molecular sequences (0.5% for COI) but are relatively low and can only be taken to indicate inter-population variability. Boeters described an unpigmented penis both in the original description (Boeters, 1970) and

in his revision (Boeters, 1988). However, we observed that the distal region of the penis contains a small black patch of pigmentation in all the populations examined.

Molecularly, *P. (C.) falkneri* represents an independent lineage within the subgenus *Corrosella*, and its relationship with the other two clades is not well defined (Figure 26). Morphological similarities with the clade comprising *P. (C.) marisolae, P. (C.) luisi, P. (C.) iruritai* and *P. (C.) andalusica* are: slender shell with same SL/SW ratio (about 1.80, Appendix III: Table 1), long penis also with the same PL/Head length ratio (about 1.30, Appendix III: Table 6) and pattern of pigmentation in renal oviduct, starting at the joining point with the bursa duct. Shared characters with *P. (C.) manueli* and *P. (C.) bareai* include: small trace of pigment on distal region of penis and shorter seminal receptacle compared with the rest of the studied species. Further, the shape and size of the shell aperture in *P. (C.) falkneri* are similar to that of *P. (C.) hydrobiopsis*.

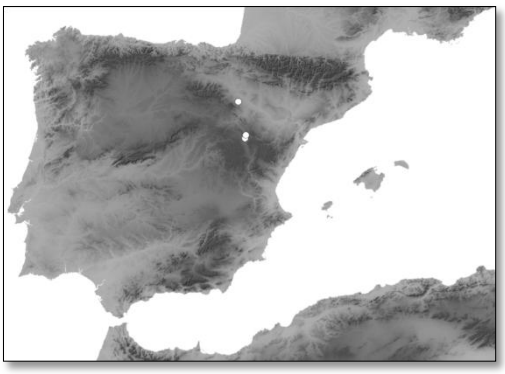
Pseudamnicola (C.) falkneri has numerous autapomorphies because it is the only species of *Corrosella* that shows: a shell as small as 2-2.6 mm, central radular tooth without cusps, six lateral cusps in radular lateral teeth, as few as 12-13 gill filaments, two female genitalia morphologies in some populations, short seminal receptacle (around 0.15 mm), prostate gland as long as almost 2 mm and RPG ratio approaching 0.5 (Appendix III: Table 7).

In our studies (Delicado *et al.*, 2012), we extended the distribution area both in Granada and in Albacete and the species was cited for the first time from the Almería province. Despite this expansion, *P. (C.) falkneri* was included as "Vulnerable" in the Red Book of the Invertebrates of Andalusia (Barea-Azcón *et al.*, 2008).

Pseudamnicola (Corrosella) hinzi Boeters, 1986

Type locality

Balsa de Vargas, Borja, Zaragoza, UTM: 30S 0509318/4194330. In the original description (Boeters, 1986: 125) type locality is described as "Zaragoza, Bulbuente". But Boeters annotated the exact locality, Balsa de Vargas, on figure captions of anatomy drawings (Boeters, 1986: 126, Figure 1-3) and of shell pictures plate (Boeters, 1986: Plate 18a). On



the other hand, Borja town council is nearer the type locality than Bulbuente.

Material Examined

Type material in Boeters, 1986: "Holotypus SMF 257 406, Paratypen SMF 257 407, BOE 599, 600 and 1240". In addition to Balsa de Vargas in Zaragoza province, this species has only been found in one locality near type locality, Cazuelas Stream, and in two localities in Teruel province (Delicado *et al.*, 2010). These four localities (Appendix II) have been studied through anatomy and molecular studies.

Diagnosis

Shell with yellowish periostracum; slightly adapically sinuate aperture; central tooth of radula with six lateral cusps; large gastric caecum; pyriform bursa copulatrix U-shaped; elongated seminal receptacle; renal oviduct black pigmented until loop; penis gradually tapering with a duct strongly undulating and with a clear patch of pigmentation on its middle region; supraoesophageal connective around four times longer than suboesophageal one.

Description

Shell ovate-conic with 4 spire whorls and height 2.75-3.5 mm (Figures 44A, B, Appendix III: Table 1), periostracum yelowhish and strongly eroded, especially in firsts whorls; protoconch approximately 450 µm width and 1.4 whorls and a nucleus around 200 µm length (Figures 44D, E); protoconch microsculpture moderately granulated (Figure 44F); convex whorls and deep sutures; peristome orthocline; inner lip of aperture thicker than outer one and partially hiding the umbilicus; the edge of peristome is slightly adapically sinuate (Figure 44C).

Operculum with 2.75 whorls and an oval muscle attachment area located near the nucleus (Figures 45A, B; Appendix III: Table 2).

Radula intermediate length (20% of total shell length) and approximately eight times longer than wide (Figure 45C, Appendix III: Table 3); around 65 rows of teeth; central tooth with a tongue-shaped median cusp and six lateral cusps (Figures 45D, E); lateral teeth with four tapered cusps shorter than the central one; inner marginal teeth contain 23 tapered cusps, shortening towards the base of tooth; outer marginal teeth with 28 tapered cusps (Figures 45D, F).

Pigmentation and anatomy. Head dark brown pigmented from snout to neck (Figure 46F); the pigmentation is clearer on neck; tentacles with medial longitudinal streak

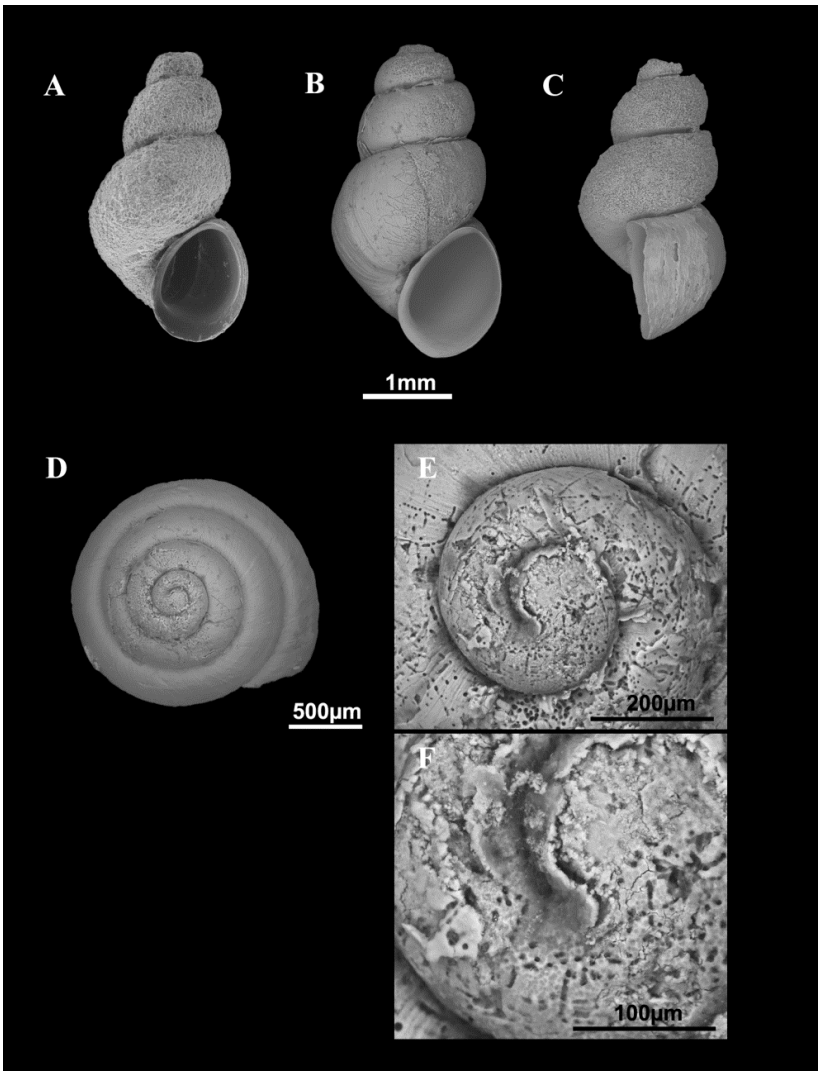


Figure 44. Shells of *Pseudamnicola (C.) hinzi*. **A, C-F.** Shells from Balsa de Vargas, Borja, Zaragoza. **B.** Shell from spring of river park in Calamocha, Teruel. **D-F.** Protoconch and microsculpture.

of brown pigment; no pigment on ocular lobes; snout as long as wide, with a strong distal lobation; foot with intermediate length and pigmentation on dorsal region. Ctenidium in the anterior region of pallial cavity and with 16-18 gill filaments taller than wide; osphradium of intermediate width under central gill filaments (Figure 46C, Appendix III: Table 4). Stomach slightly longer than wide and posterior chamber a little larger than the anterior one (Figure 46E, Appendix III: Table 4); large gastric caecum; style sac longer than wide and surrounded by intestine, presenting this last one small path of brown pigment.

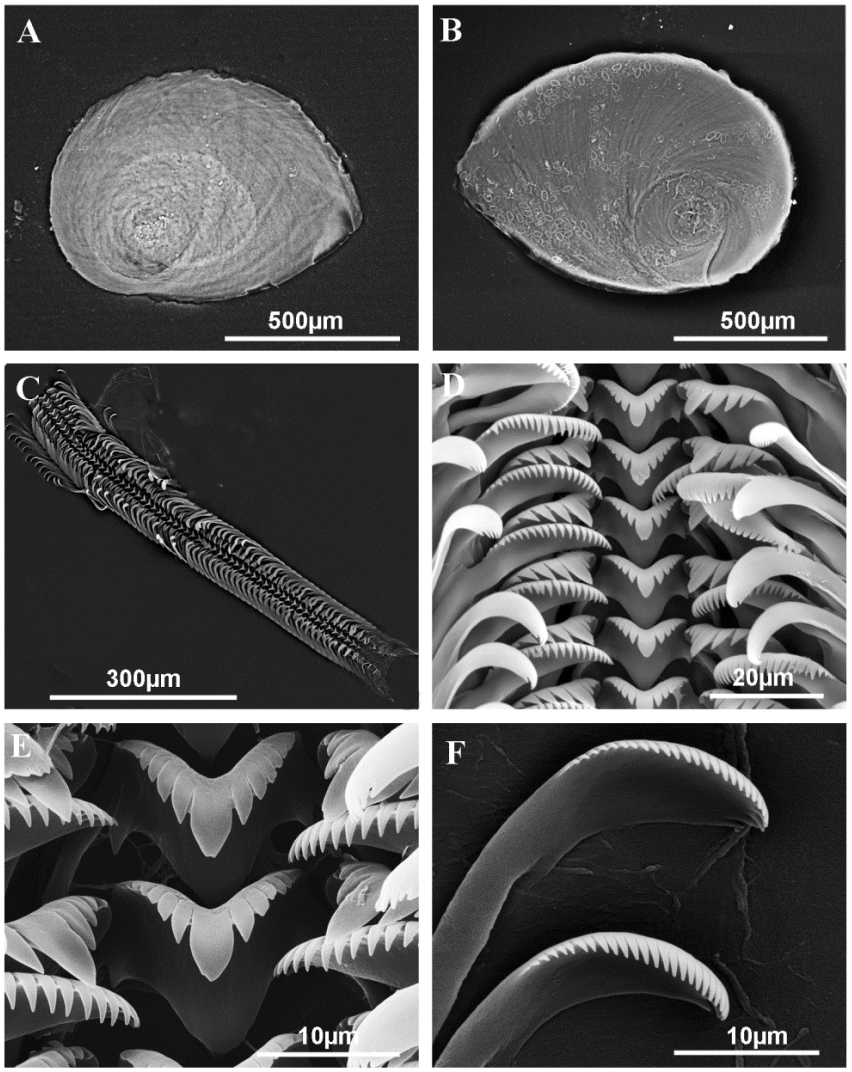


Figure 45. Operculum and radula of *Pseudamnicola (C.) hinzi* from Balsa de Vargas, Borja, Zaragoza. **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central tooth. **F.** Outer marginal teeth.

Female genitalia with a pallial oviduct containing a capsule gland 1/3 longer than albumen gland (Figure 46H; Appendix III: Table 5); from pyriform to elongate bursa copulatrix folded in U-shaped with a duct about three times shorter than bursa length; renal oviduct black pigmented until loop; pyriform seminal receptacle with a short duct (Figure 46I); it lays on renal oviduct slightly above the point where the bursal duct joins the renal oviduct.

Male genitalia contains a prostate gland bout three times longer than wide (Figure 46D, Appendix III: Table 6); penis gradually tapering with a duct strongly

undulating and some folds and a clear path of brown pigment on its middle region; the base is attached to central area of the head (Figures 46F, G).

Nervous system dark brown pigmented; cerebral ganglia equal in size and darker than the rest of ganglia and connectives; supraoesophageal connective approximately six times longer than suboesophageal one (Figures 46A, B; Appendix III: Table 7). RPG ratio is 0.58 in mean (elongated).

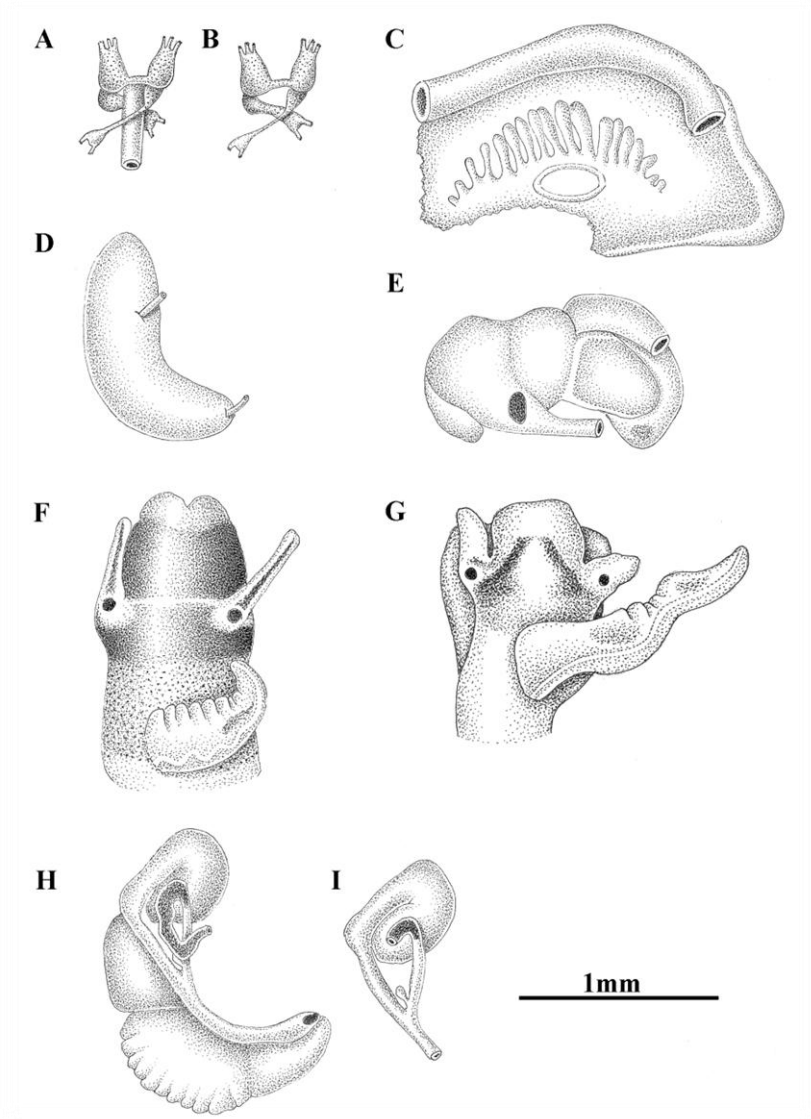


Figure 46. Anatomy of *Pseudamnicola (C.) hinzi* from Balsa de Vargas, Borja, Zaragoza (**A**-**F**, **H**, **I)** and from spring of river park in Calamocha, Teruel (**G**). **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Prostate gland. **E.** Stomach. **F**, **G.** Head of a male and penis. **H.** Female genitalia. **I.** Bursa copulatrix and seminal receptacle.

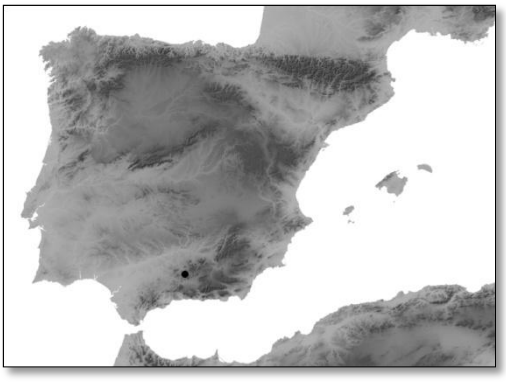
Remarks. Through the identification of this species collected from type locality, molecular and morphological studies reveal that *P. (C.) hinzi* actually does not occur in Pozo Azul (Burgos province), as Boeters declared in 1988. This population and the ones from nearby areas belong to the species *P. (C.) navasiana*, hence the distribution area of *P. (C.) hinzi* is reduced to some localities in the northeastern Iberian Peninsula. Despite inhabiting a small territory, some anatomical differences exist within the species: populations from Teruel province show longer shells and penis (Figures 46A, B) however, they share a similar shape in female genitalia, nervous system and stomach with populations from Zaragoza province.

Genetic divergence between this species and species from the north of the Iberian Peninsula, *P. (C.) navasiana* and *P. (C.) hauffei*, comes to be about 9.2% for COI, 4.2% 16S and 0.4% 28S; and the differences between *P. (C.) hinzi* and the rest of *Corrosella* species from the South, are around 9.4% in COI, 7.6% in 16S and 0.4% for 28S (See Appendix IV: Table 1). Even occurring in the same area than the species from the north, *P. (C.) hinzi* shares some characteristics with them but, besides, some other ones from southern species. Thus, characters like OL/OW ratio less than 1.5 (Appendix III: Table 2), cylindrical bursa copulatrix, seminar receptacle shorter than 0.18 mm (Appendix III: Table 5) and a nervous system from moderately concentrated to elongate, are common between the northern species, *P. (C.) manueli*, *P. (C.) bareai* and *P. (C.) hinzi*. Nevertheless, this last one also partakes off some traits with the species *P. (C.) iruritai*, *P. (C.) ballestae* n. sp., *P. (C.) marisolae* and *P. (C.) luisi* such as: more conic shell shape (SL/SW ratio around 1.75, Appendix III: Table 1) and gradually tapering penis with attachment area to head central.

Pseudamnicola ? (Corrosella) hydrobiopsis Boeters, 1999

Type locality

La Carmonilla spring in Loja, Granada, Spain, UTM: 30S 399062/4113653.



Type material

The species is described based on the following type material: "Holotype (NNM 59145) and two paratypes (NNM 59146) ex BOE 1399" (Boeters 1999).

Material examined

After exhaustively exploring the type locality and surrounding areas (according to the standard and Bou-Rouch methods used to collect the original shells) the species could not be found. The holotype, a dry shell, was borrowed from RMNH and examined and measured. One paratype was measured on the picture in the original description.

Description

Original description in Boeters (1999) (figures 1, 2). Since no live specimens were found, the description below only includes shell features.

Shell shape turbinated, whitish as a result of erosion, height about 3.5 mm (Appendix III: Table 1), 5.5 shell whorls (Figure 47A); in non-eroded spire whorls, longitudinal thin stripes can be observed; tip of shell, therefore protoconch, eroded (Figure 47C); no deep suture between spire whorls and oblique; peristome frontal, complete, oval, with a pointed superior edge; thin outer lip and thicker inner lip which partially hides the umbilicus because of contact with body whorl; in lateral view, peristome is not straight since it is broken (Figure 47B).

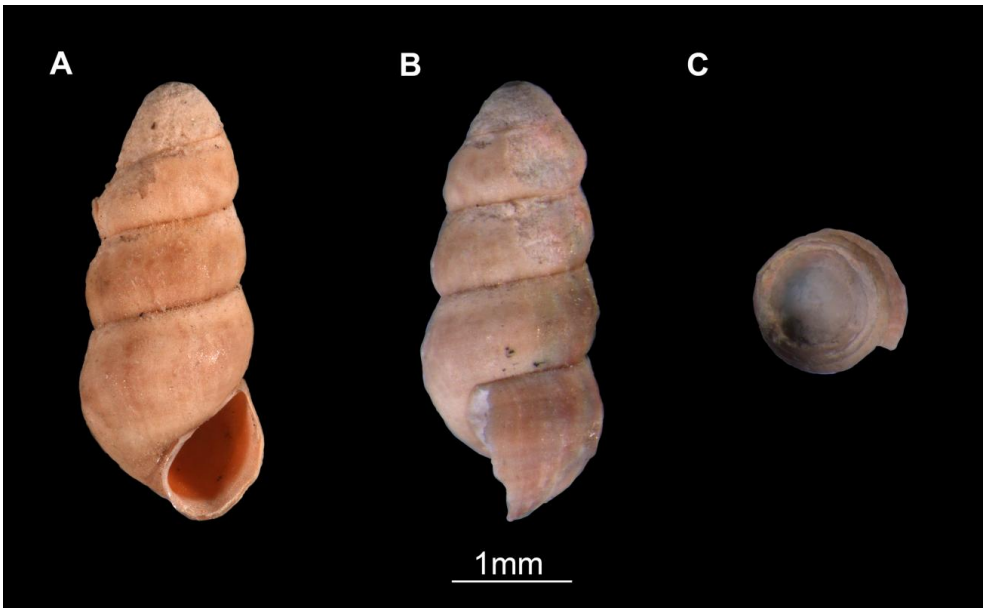


Figure 47. Holotype of *Pseudamnicola? (C.) hydrobiopsis* (NNM 59145).

Remarks. This species was originally described based only on three shells found through "sondage Bou-Rouch" (Boeters, 1999). Ten years later this sampling

method was repeated at the type locality, but no shells were found either there or at any surrounding locality. The description of the species therefore remains incomplete.

The type material and original description suggest that shell shape resembles species of *Hydrobia* more than *Pseudamnicola*. In fact, the name of the species refers to similar shell characters to those seen in *Hydrobia* (Boeters, 1999). Given the lack of anatomical data and its similar shell features to the genus *Hydrobia*, it might be more appropriate to include it in this genus, or in *Moitessieria* Bourguignat, 1863. However, until a live specimen is found, its generic placement remains uncertain.

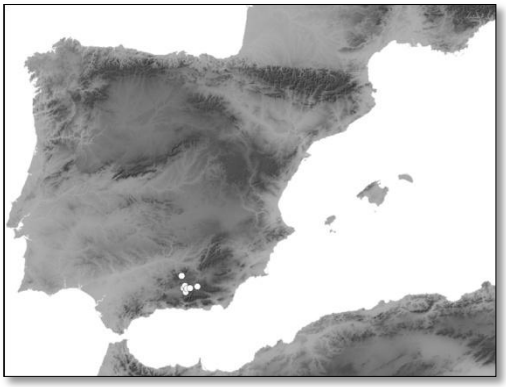
One of the differentiating characters of so called *P. (C.) hydrobiopsis* is a turbinated shell and 5.5 spire whorls, differing from all the *P. (Corrosella)* species discovered to date. *Pseudamnicola (C.) iruritai* inhabits a spring near the type locality of *P. (C.) hydrobiopsis*, but the shell of *P. (C.) iruritai* differs in its proportions despite being of similar size. The body whorl and peristome of *P. (C.) iruritai* are larger and have a thicker inner lip completely hiding the umbilicus, whereas the umbilicus of *P. (C.) hydrobiopsis* is slit-like. Further, the spire whorls in *P. (C.) hydrobiopsis* are wider and bear more oblique suture.

Pseudamnicola (C.) luisi and *P. (C.) marisolae* are the closest species geographically, but are larger, more conical, with a larger aperture and umbilicus. *Pseudamnicola (C.) falkneri* and *P. (C.) bareai* inhabit the same province but further away, and are smaller, also more conical, with shorter and narrower spire whorls and a thicker inner lip in the peristome.

***Pseudamnicola (Corrosella) luisi* Boeters, 1984**

Type locality

Lapeza, Granada VG 72 (Boeters 1984).



Material examined

Type material. Holotype SMF 256 391, Paratype BOE 224, Slg Falkner and RMNH Leiden, Slg. Altimira (Boeters, 1984).

Topotypes. Boeters (1984) only cited Lapeza, Granada as the type locality. We found the species in La Gitana spring in La Peza (= Lapeza), Granada, UTM: 30S

047415/412475. This is most probably the type locality because this spring is the freshwater habitat closest to La Peza within the 10 × 10 km VG72 UTM grid mentioned by Boeters (1984), although he used the Military Grid Reference System. The description of this species has therefore been based on this topotypical material

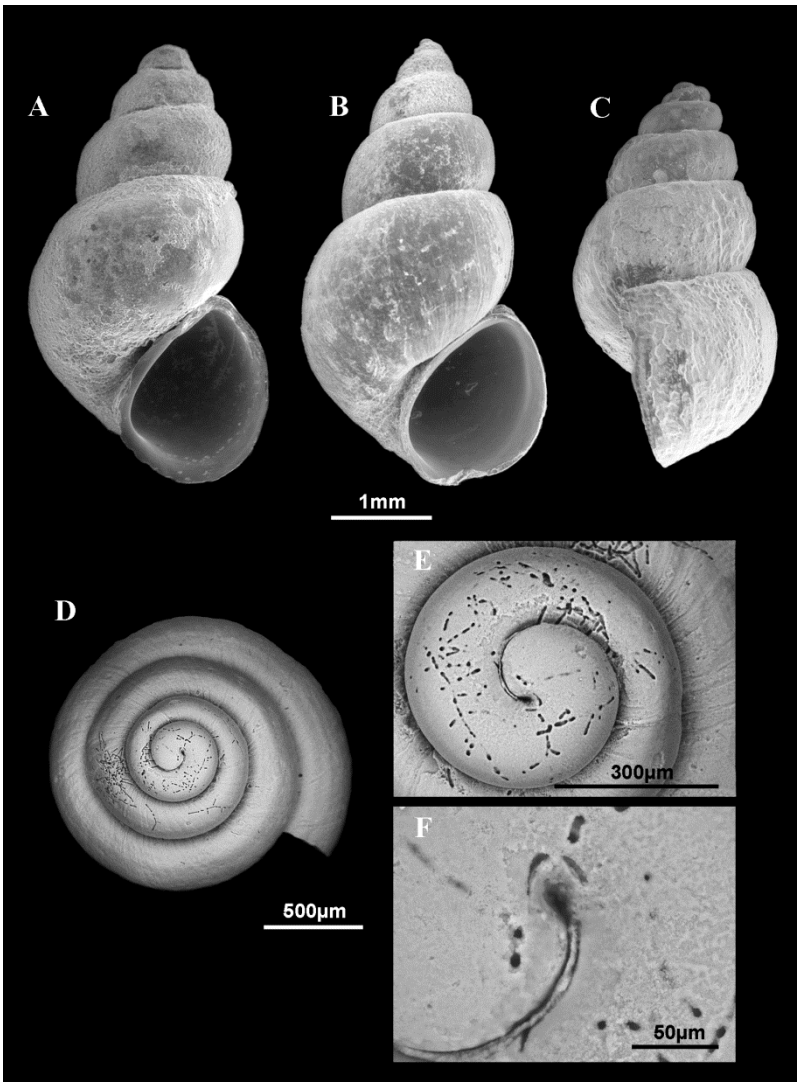


Figure 48. Shells of *Pseudamnicola (C.) luisi*. **A**, **C-F.** Shells from La Gitana spring in La Peza, Granada. **B.** Shell from Polvorista stream, Quéntar, Granada. **D-F.** Protoconch and detail of protoconch microsculpture.

collected in different years and deposited in the MNCN collections under MNCN 15.05/49147, MNCN 15.05/49148, MNCN 15.05/49149, MNCN/AND 34827-34830, MNCN/ADN 34827-34830, MNCN 15.05/49150.

Other populations examined. In addition to La Gitana spring, this species has only been found in a few localities in the centre of Granada province (Appendix II). Boeters (1984, 1988) cited the species also in Almería province (WF 79 el Maltés, Níjar, Rijksmuseum van Natuurlijke Historie Leiden, Slg. Altimira, *Pseudamnicola brevispira*), although he included no drawings of specimens from this province. We have found no specimens of this species in Almería. Specimens of all localities mentioned in the following section were also morphologically examined.

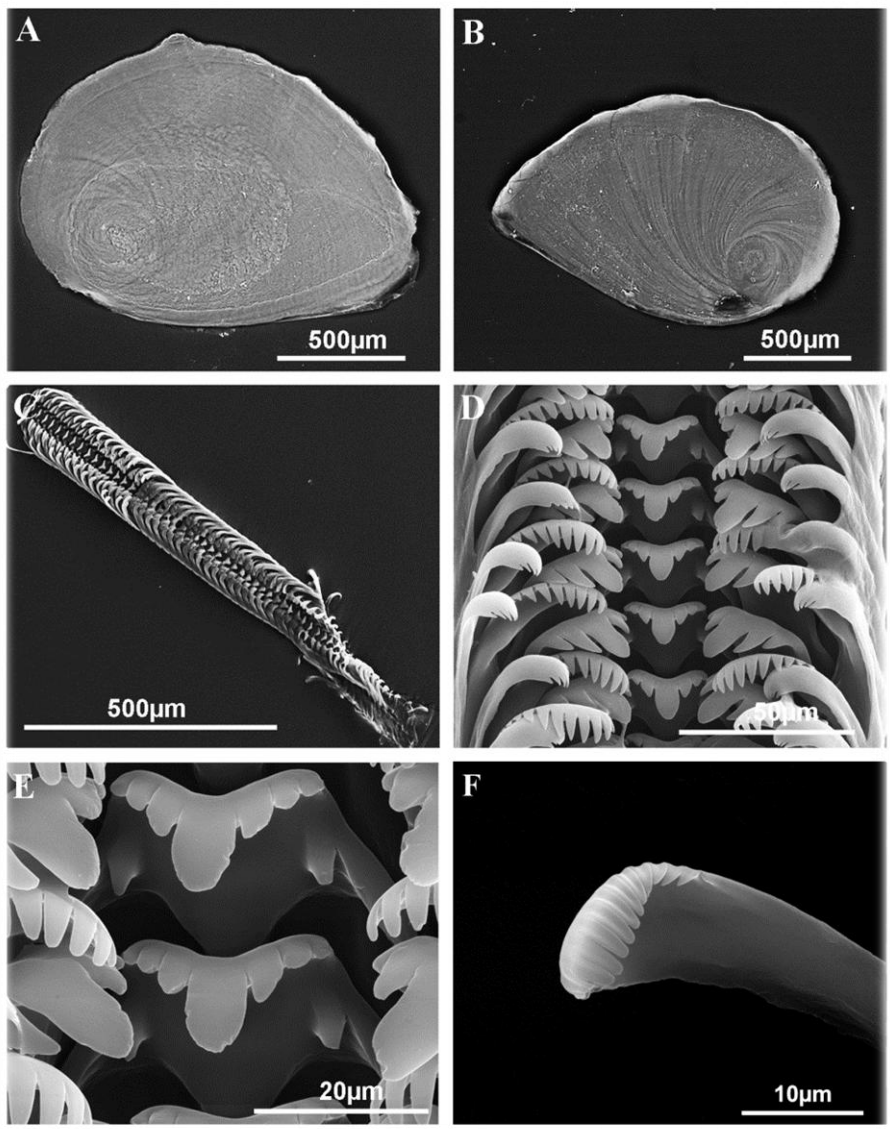


Figure 49. Operculum and radula of *Pseudamnicola (C.) luisi* from La Gitana spring in La Peza, Granada. **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central tooth. **F.** Detail of an outer marginal tooth.

Material examined for morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Tables 1-7) taken from topotypes from La Gitana spring in La Peza, Granada. Males and females examined were collected in March, May and September.

Diagnosis

Shell with whitish or greyish periostracum; tall ctenidial filaments; central tooth of radula with three wide lateral cusps at each side; black pigmentation on anterior chamber of stomach and intestine; pyriform bursa copulatrix U-shaped; elongated seminal receptacle; renal oviduct black pigmented until loop; slender penis with a large patch of pigmentation on its distal region; nervous system black pigmented, ganglia darker than connectives; supraoesophageal connective at least four times longer than suboesophageal.

Description

Shell ovate-conic with 4-5.5 spire whorls, height 3.5-5 mm (Figures 48A, B, D; Appendix III: Table 1), periostracum whitish or greyish; protoconch approximately 500 μm in width with 1.5 whorls and a nucleus around 170 μm long (Figures 48D, E); protoconch microsculpture moderately granulated (Figure 48F); body whorl about two-thirds total length and penultimate whorl relatively taller than previous ones; teleoconch whorls moderately convex with a deep suture; inner lip of aperture thicker than outer lip and partially hiding the umbilicus; peristome margin simple, straight (Figure 48C).

Operculum with around 3.5 spire whorls and muscle attachment area oval located near the nucleus (Figures 49A, B; Appendix III: Table 2).

Radula intermediate length (20% total shell length) and approximately eight times longer than wide (Figure 49C, Appendix III: Table 3); bears some 51 rows of teeth; central tooth with a tongue-shaped median cusp and three wide lateral cusps at each side slightly sharpening towards median cusp (Figures 49D, E); lateral teeth with two tapered cusps at each side of central tongue-shaped cusp; inner marginal teeth have 12 sharp cusps, shortening towards the base of tooth; outer marginal teeth with 15 tapered cusps (Figures 49D, F).

Pigmentation and anatomy. Head dark brown pigmented from snout to neck (Figure 50F); pigmentation is clearer on neck; tentacles also brown pigmented but not ocular lobes; snout as long as wide, with medial lobation; foot of intermediate length, pigmented in dorsal region. Ctenidium in the anterior region of pallial cavity with 20-24 large gill filaments taller than wide; osphradium of intermediate width under central gill filaments (Figure 50C, Appendix III: Table 4). Stomach slightly longer than wide with two chambers equal in size (Figure 50E); the anterior chamber could

display a small patch of black pigmentation; long gastric caecum; style sac longer than wide surrounded by black pigmented intestine (Appendix III: Table 4).

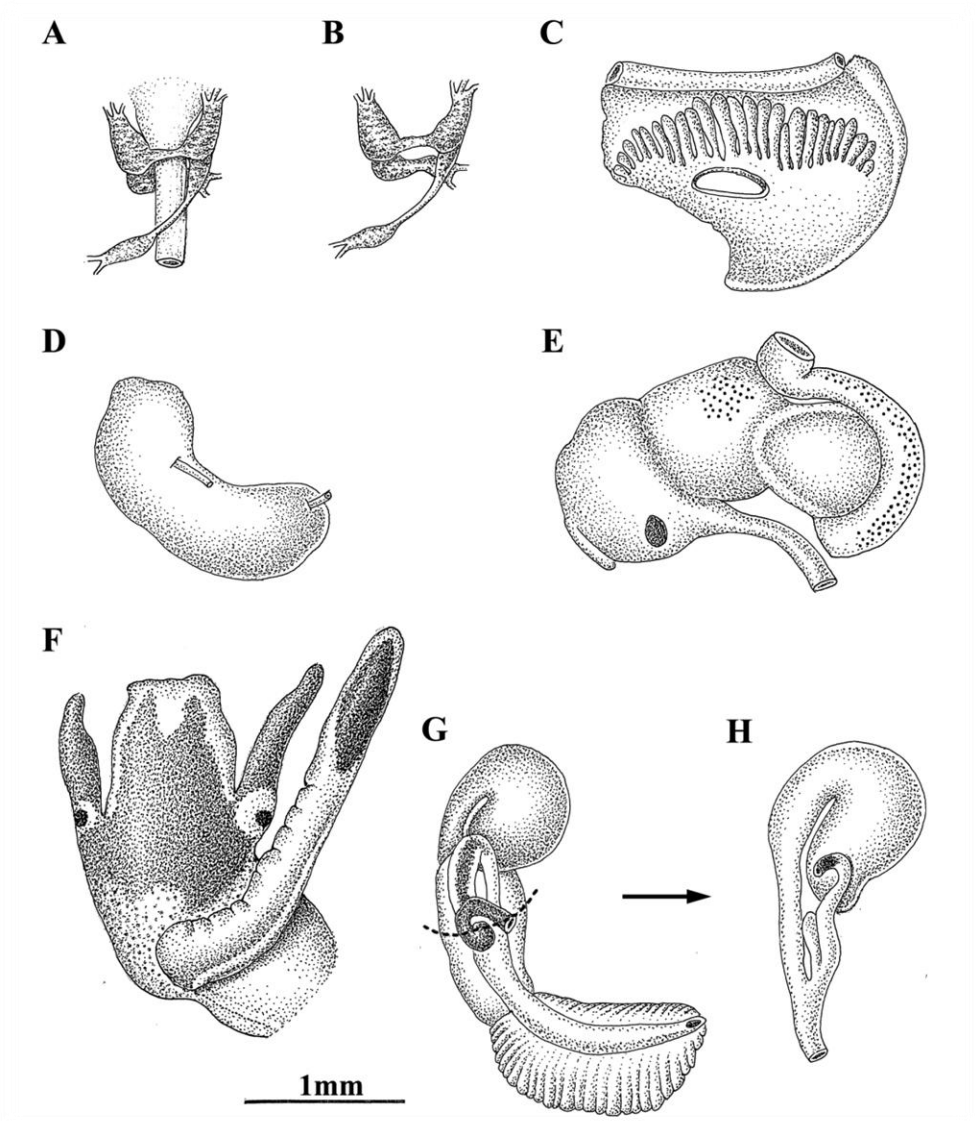


Figure 50. Anatomy of *Pseudamnicola (C.) luisi* from the type locality. **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Prostate gland. **E.** Stomach. **F.** Head of a male and penis. **G.** Female genitalia. Dotted line shows the pallial wall. **H.** Bursa copulatrix and seminal receptacle.

Female genitalia with a slender pallial oviduct containing two glands approximately equal in size (Figure 50G; Appendix III: Table 5); capsule gland more transparent than albumen gland in the specimens studied; bursa copulatrix pyriform, long, folded and U-shaped with a duct less than 50% bursa length; renal

oviduct white straight from the insertion point of bursal duct to where it begins to fold and black pigmented making a simple loop, hereafter undulating; seminal receptacle elongated with short duct (Figure 50H) joining renal oviduct slightly above the point where the bursal duct joins the renal oviduct; there is no contact between distal end of bursa copulatrix and distal end of seminal receptacle.

Male genitalia bears a two or three times longer than wide prostate gland (Appendix III: Table 6) with an efferent duct entering the medial region and deferent duct emerging at its anterior edge (Figure 50D); penis, long slender, with a blunt distal end and a large patch of pigmentation distally; base of intermediate width attached to the central head area with some folds in its middle region (Figure 50F); penial duct scarcely visible running straight close to the outer penis margin.

Nervous system black pigmented, but ganglia darker than connectives and commissures; cerebral ganglia equal in size; supraoesophageal ganglion around two times longer than suboesophageal and supraoesophageal connective approximately five times longer than suboesophageal (Figures 50A, B; Appendix III: Table 7). Mean RPG ratio 0.41 (moderately concentrated).

Remarks. Among the *Pseudamnicola*, *P. (C.) luisi* is the largest species, measuring about 5 mm in height. All specimens showed signs of erosion, not only on the protoconch as in other species, but all over the shell surface.

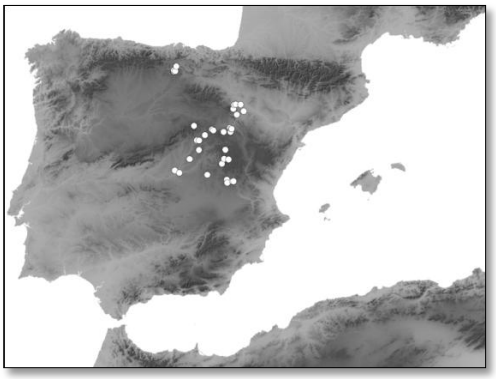
Pseudamnicola (C.) marisolae, P. (C.) iruritai and *P. (C.) andalusica* are sister species, belonging to the same clade as *P. (C.) luisi* (Figure 76) but not as large. These four species share slender shells, a high SL/SW value (Appendix III: Table 1), long penis with a large patch of pigmentation, long prostate gland and one seminal receptacle with a short duct. *Pseudamnicola (C.) marisolae* is molecularly the closest and also biogeographically the nearest species to *P. (C.) luisi* (Appendix IV: Table 1). The two species share characteristics such as: same number of cusps on lateral and inner marginal teeth, same number of gill filaments, same length of connectives in nervous system and similar shape of penis and bursa copulatrix. *Pseudamnicola (C.) luisi* differs from the rest of the species of this clade in its granulate protoconch microsculpture, long gill filaments, longer pallial oviduct, larger penis and shell aperture than the rest of species, lack of pigmentation of distal section of renal oviduct and seminal receptacle and the lowest RPG ratio. All these characters serve also to differentiate *P. (C.) luisi* from *P. (C.) falkneri*, *P. (C.) manueli* and *P. (C.) bareai*, these species showing, as well as smaller shells with a lower SL-LBW value (Appendix III: Table 1), different lateral teeth formula [six lateral cusps in *P. (C.) falkneri*, three in *P. (C.) manueli* and four in *P. (C.) bareai*], fewer gill filaments (12-19), a not so slender penis with a smaller patch of pigmentation and shorter seminal receptacle without duct, approximately half the length of that in *P. (C.) luisi*.

Because of its restricted distribution area, *P. (C.) luisi* has been included as "Near threatened, NT" in the Red Book of Invertebrates of Andalusia (Barea-Azcón *et al.*, 2008).

***Pseudamnicola (Corrosella) navasiana* (Fagot, 1907)**

Amnicola navasiana Fagot 1907, *Bol. Soc. aragon. Cienc. nat*., 6:158.

Pseudamnicola (Corrosella) navasiana (Fagot): Boeters 1988: *Arch. Moll.*, 118: 203, 205, figs. 65, 71, 87, 286, pl. 2 fig. 20.



Type locality

Neither type material nor the exact type locality is reflected in the original description (Fagot, 1907).

Type material. Unknown.

Material Examined

Topotypes. Boeters (1988) only cited the species in Bulbuente, Zaragoza (BOE 1 239 Ex. Hinz). We have found also the species in Fonnueva Spring in Bulbuente, Zaragoza, UTM: 30T 0634067/4630860.

Other populations examined. In addition to type locality a high number of localities have been found among North-East quadrant of the Iberian Peninsula. All of them are gathered in Appendix II.

Material Examined for Morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Table 1) correspond to topotypes from Fonnueva spring in Bulbuente, Zaragoza. Male and females studied and measured were collected in the following months: March, May and September.

Diagnosis

Shell with whitish or yellowish periostracum and about 4 spire whorls; inner peristome thicker than outer peristome; central and lateral teeth of radula with five and three lateral cusps respectively; dark melanic pigment on external surface of the body; style sack surrounded by black pigmented intestine; penis gradually tapering attached close to right eye and with a patch of pigmentation from the medium region to the tip; pyriform bursa copulatrix J-shaped; black pigmented nervous system; supraoesophageal connective about five times longer than suboesophageal.

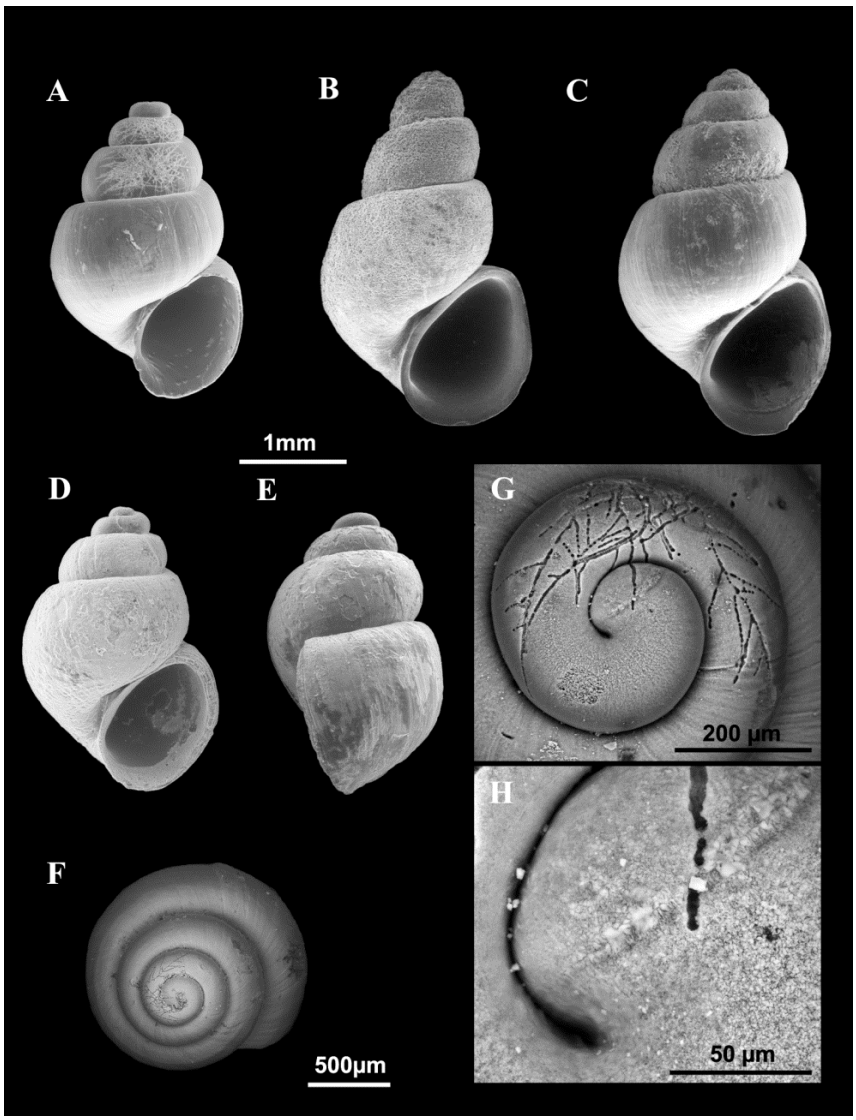


Figure 51. Shells of *Pseudamnicola (C.) navasiana*. **A, B, F-H.** Shells from Fonnueva Spring, Bulbuente, Zaragoza. **C.** Shell from María Spring, Ontígola, Toledo. **D-E.** Shells from Pozo Azul, Covanera, Burgos. **F-H.** Protoconch and detail of protoconch microsculpture.

Description

Shell ovate-conic (Figures 51A-D), whitish or greyish with 3.5-4.5 spire whorls and a height of around 3-4 mm (Appendix III: Table 1); protoconch approximately 450 µm width and 1.5 whorls and a nucleus around 200 µm length (Figures 51F, G); protoconch net-shaped grooved (Figure 51H); last body whorl about 2/3 of total length; convex whorls and deep sutures; inner lip wider than outer and partially hides the umbilicus; the edge of peristome is simple and straight (Figure 51E).

Operculum with 2.5 spire whorls approximately (Figures 52A, B; Appendix III: Table 2) and a muscle attachment area in the internal side oval located near the nucleus.

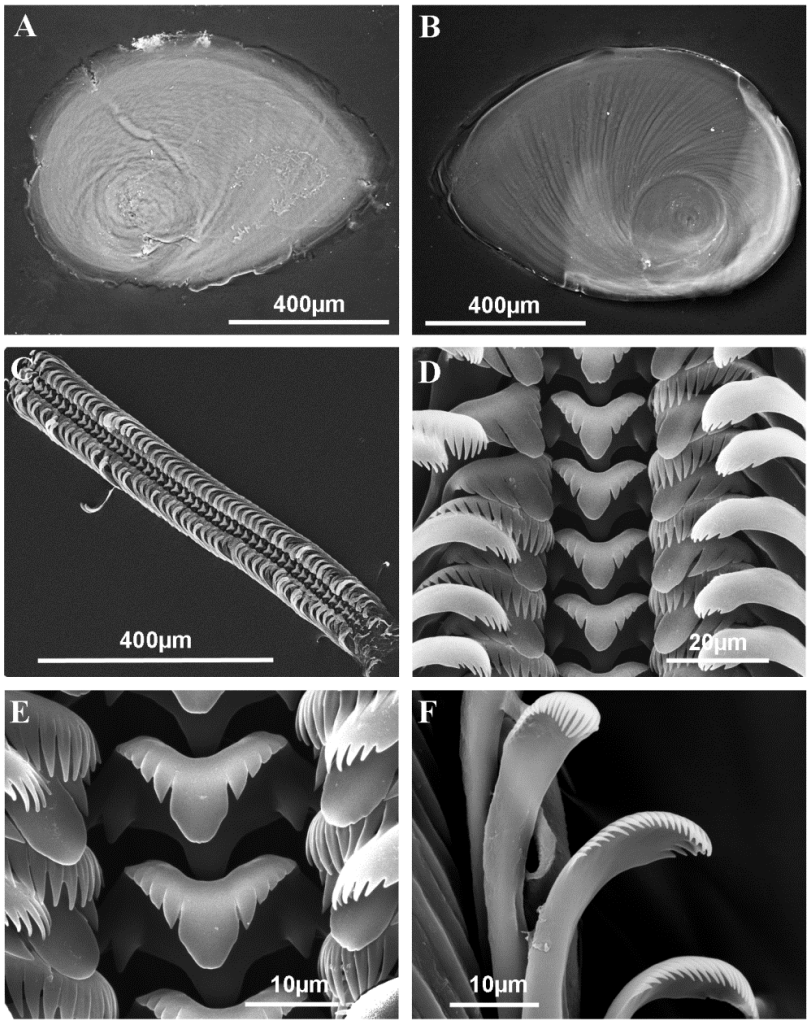


Figure 52. Operculum and radula of *Pseudamnicola (C.) navasiana* from Fonnueva Spring, Bulbuente, Zaragoza. **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central tooth. **F.** Detail of an outer marginal tooth.

Radula intermediate length (25% of total shell length) and approximately eight times longer than wide (Figure 52C, Appendix III: Table 3); it has around 55 rows of teeth; central tooth with a tongue-shaped central cusp and five lateral cusps at each side, slightly sharpening towards central one (Figures 52D, E); lateral teeth with three or four tapered lateral cusps; inner marginal teeth contain about 20 sharp cusps, shortening towards the base of tooth; outer marginal teeth with around 22 tapered cusps (Figures 52D, F).

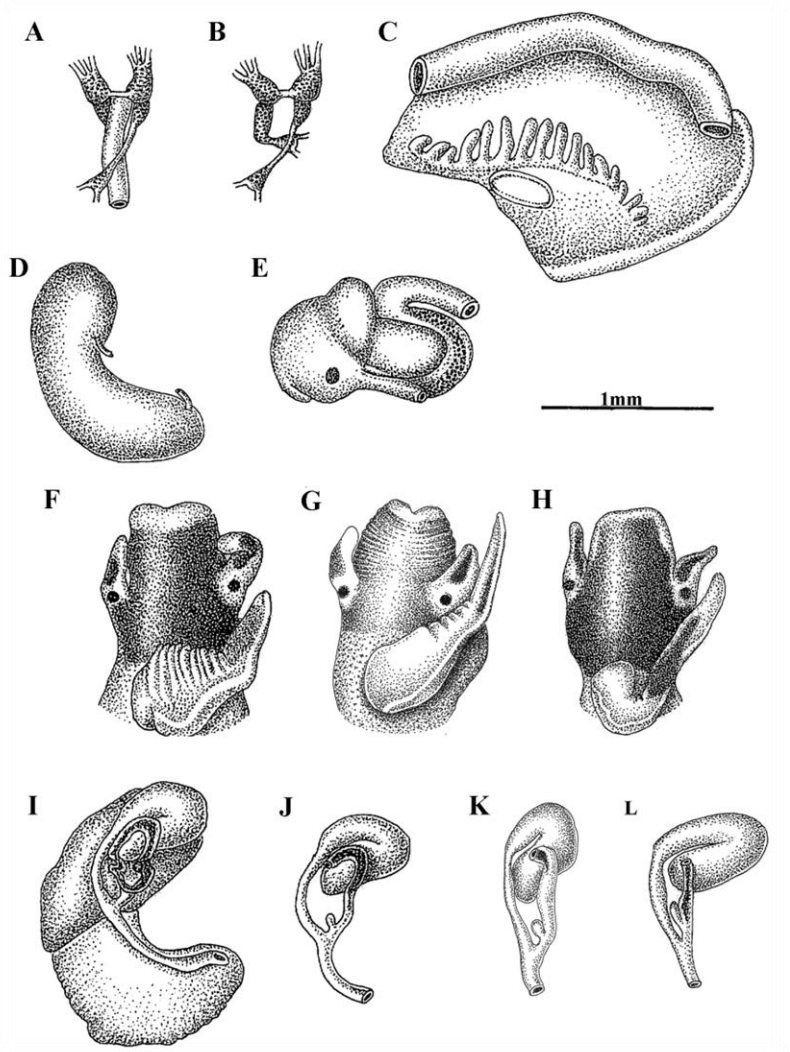


Figure 53. Anatomy of *Pseudamnicola (C.) navasiana* from Fonnueva Spring, Zaragoza (**A-F, I-J**). **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Prostate gland. **E.** Stomach. Head of a male and penis from: Fonnueva spring (**F**), Maria spring, Ontígola, Toledo (**G**), Pozo Azul, Burgos (**H**). Female genitalia from Fonnueva spring (**I**). Bursa copulatrix and seminal receptacle from: Fonnueva spring (**J**), Maria spring, Ontígola, Toledo (**K**), Pozo Azul, Burgos (**L**).

Pigmentation and anatomy. External surface intensely brown pigmented; the pigmentation is clearer on neck; brown pigment on tentacles too, but not on ocular lobes (Figure 53D); snout as long as wide, with medial lobation; foot with intermediate length and dorsal pigmentation. Ctenidium in the anterior region of pallial cavity and with 15-20 gill filaments; osphradium of intermediate width and opposite approximate middle of ctenidium (Figure 53C, Appendix III: Table 4). Stomach slightly longer than wide and with two similar-size cameras (Figure 53F); style sac longer than wide surrounded by black pigmented intestine (Appendix III: Table 4).

Female genitalia with a capsule gland one-third longer than albumen gland (Figure 53G; Appendix III: Table 5); pyriform bursa copulatrix J-shaped with a shorter bursa duct; renal oviduct straight and black pigmented from the joining point of bursal duct joins to the renal oviduct folds, from which oviduct makes one or two vertical loops; pyriform seminal receptacle without or with a short duct (Figure 53H) joining renal oviduct slightly above the point where the bursal duct joins.

Male genitalia contains a prostate gland around three times longer than wide (Figure 53E; Appendix III: Table 6); penis gradually tapering with a patch of pigmentation in its middle distal region; it is attached to central head area with some folds in the middle region (Figure 53F); penial duct running straight close to the external margin of penis.

Nervous system black pigmented, darker on ganglia than connectives and commissures; cerebral ganglia with equal size approximately; supraoesophageal connective about four or five times longer than suboesophageal one (Figures 53A, B; Appendix III: Table 7). RPG ratio is 0.42 of mean (moderately concentrated).

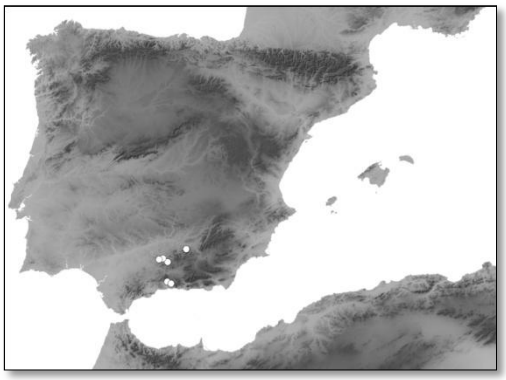
Remarks. *P. (C.) navasiana* is the most broadly extended species of *Pseudamnicola* from Iberian Peninsula. As a result of this study, we can confirm that not only does this species occur in one spring in Bulbuente, Zaragoza province (Boeters, 1988), but also in many freshwater localities along high and middle courses of Ebro and Tagus rivers. The populations from Castilla-La Mancha region in Spain cited for the species *P. (C.) luisi* and *P. (P.) gasulli* and those from Guadalajara province cited for *P. (C.) falkneri* (Bragado *et al.*, 2010) may therefore belong to *P. (C.) navasiana*.

Such wide distribution, comparing to the limited territory of other hydrobiids, might be the reason for its morphological and molecular variability (Appendix IV: Table 1) among its populations. Most of this variation is reflected in shape and size of shell (Figure 51 and Appendix III: Table 1) and in size of penis (Figure 53F-H) and bursa copulatrix (Figure 53I-L). Genetic divergences within the species are around 1.2% for COI, 0.3% for 16S and less than 0.1% for 28S (Appendix IV: Table 1).

Pseudamnicola (Corrosella) andalusica Delicado, Machordom and Ramos, 2012

Type locality

La Salud spring in Albanchez de Mágina, Jaén, Spain, UTM: 30S 0459004/ 4181366.



Type material

Holotype MNCN 15.05/53713a (ESEM preparation, Figure 54A), paratypes MNCN 15.05/53713b (96% ethanol, Figures 54C-G, 55) and MNCN/ADN 39781-39787 (96% ethanol), JM.B. and I.B., 10 May 2009; I.B., 16 February 2010, MNCN 15.05/53714 (70% ethanol, Figure 56).

Other populations examined

This species was found at other sites in Málaga and Córdoba provinces. All the localities are shown in Appendix II.

Etymology

The name *andalusica* is a Latin adjective that refers to the Spanish southern region of Andalusia, where this species lives.

Diagnosis

Shell slender, peristome with inner lip thicker than outer; radula with four lateral cusps in central tooth and three in lateral tooth; intestine pigmented; female genitalia with a pyriform J-shaped bursa copulatrix and an elongated seminal receptacle with a short pigmented duct; renal oviduct brown pigmented; long penis with wide base, a large patch of black pigment on distal region and folds in the middle section; nervous system brown pigmented with dispersed pigment granules; supraoesophageal connective around four times longer than suboesophageal.

Description

Shell yellowish periostracum, with four to five spire whorls (Figures 54A, B, Appendix Table 1), height 3.60-3.20 mm; protoconch approximately 475 μm wide, with 1.3 whorls and nucleus around 200 μm long (Figures 54D, E); protoconch microsculpture slightly grooved (Figure 54F); body whorl about two-thirds of total length; peristome frontal, complete, oval, with thin outer lip and thicker inner lip which partially hides the umbilicus; edge of peristome simple and straight (Figure 54C).

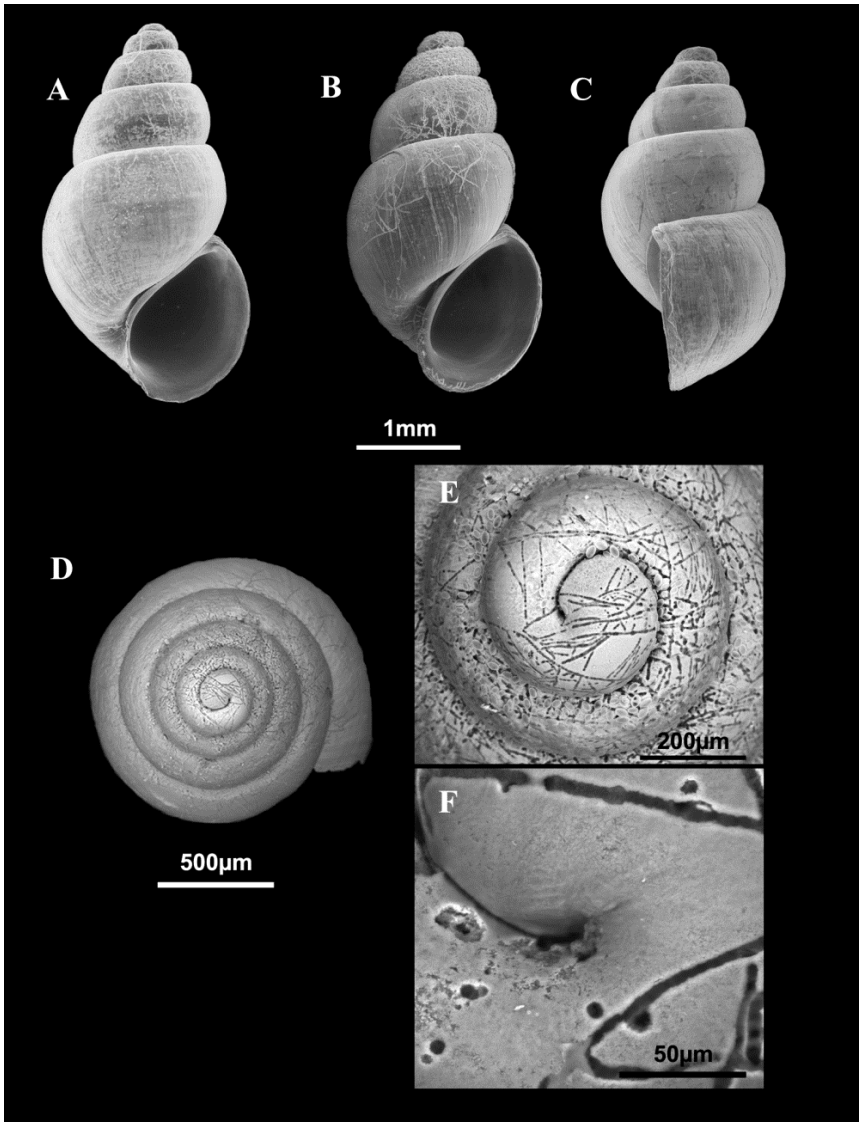


Figure 54. Shells of *Pseudamnicola (C.) andalusica*. **A**, **C**-**G.** Shells from La Salud spring in Albanchez de Mágina, Jaén (type locality). **A.** Holotype. **B.** Eduardo spring, Alcaucín, Málaga. **C**-**G.** Paratypes, protoconch and detail of its microsculpture.

Operculum with around three spire whorls (Figure 55A, B, Appendix III: Table 2) and oval muscle attachment on internal side near the nucleus.

Radula around 45 rows of teeth; intermediate length (23% total shell length) and around nine times longer than wide (Figure 55C, Appendix III: Table 3); trapezoidal central tooth with a long central cusp and four tapered lateral cusps, slightly sharpening towards central one (Figures 55D, E); lateral teeth with three sharp lateral cusps, the last one very short; inner marginal teeth contain 13 sharp cusps, shortening towards the base of tooth; outer marginal teeth with around 15 tapered cusps (Figure 55D, F).

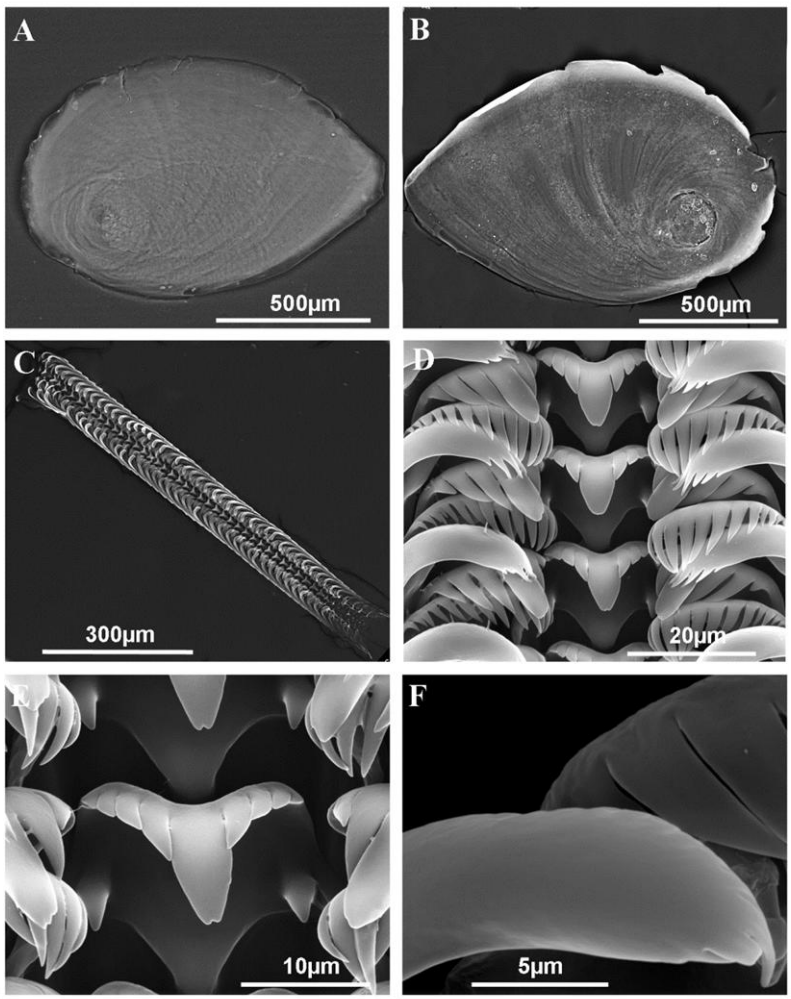


Figure 55. Operculum and radula of *Pseudamnicola (C.) andalusica* from La Salud spring in Albanchez de Mágina, Jaén (type locality). **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central teeth. **F.** Detail of an outer marginal tooth.

Pigmentation and anatomy. Head dark brown pigmented from snout to neck (Figure 56D); brown pigment also on tentacles but ocular lobes and tip nonpigmented; snout as long as wide, with medial lobation; foot of intermediate length, pigmented dorsal region. Ctenidium composed of around 20 well-developed gill filaments longer than wide situated in middle region of pallial cavity; ellipsoidal osphradium under central gill filaments (Figure 56C, Appendix III: Table 4). Stomach slightly longer than wide (Figure 56E); long gastric caecum; style sac longer than wide, with clear brown pigment and surrounded by brown pigmented intestine (Appendix III: Table 4).

Female genitalia (Figure 56G, Appendix III: Table 5) with albumen gland smaller than capsule gland and transparent; capsule gland contains two regions, the anterior one being more transparent; bursa copulatrix pyriform J-shaped with a duct slightly shorter than bursa length; elongated seminal receptacle with short brown pigmented duct (Figure 56H) linked to renal oviduct above the insertion of bursal duct; renal oviduct straight with clear brown pigment from the joining point of bursal duct to the oviduct fold, from which the oviduct is dark brown pigmented and makes two or three loops thereafter.

Male genitalia with a prostate gland around three times longer than wide (Appendix III: Table 6), vas efferens enters the medial region and vas deferens emerges at its anterior edge (Figure 56E); penis long, slender, with a blunt distal end and a large grey patch of pigmentation in its middle-distal region; attached to the central head area with some folds in its middle region (Figure 56D); penial duct running straight close to the external side of penis.

Nervous system brown pigmented, darker on ganglia than connectives and commissures, with dispersed pigment granules; cerebral ganglia and pleural ganglia approximately equal in size; supraoesophageal ganglion slightly longer than suboesophageal, and supraoesophageal connective approximately four times longer than suboesophageal (Figures 56A, B, Appendix III: Table 7). RPG ratio 0.43 on average (moderately concentrated).

Remarks. Morphological variability between the two populations examined is mostly found in the female genitalia. Females from Eduardo Spring (Málaga) have a wider and shorter bursal duct than those from the type locality and a shorter seminal receptacle without pigment. However, the molecular results indicate relatively low genetic divergence between them (1.8%) (Appendix IV: Table 1). These characters are therefore insufficient to consider them different species.

Among the species belonging to the same clade, *P. (C.) andalusica* shares most synapomorphies with *P. (C.) marisolae*, such as shell size and shape (Figures 56 and 25), a slender and pigmented seminal receptacle and a slender penis with a

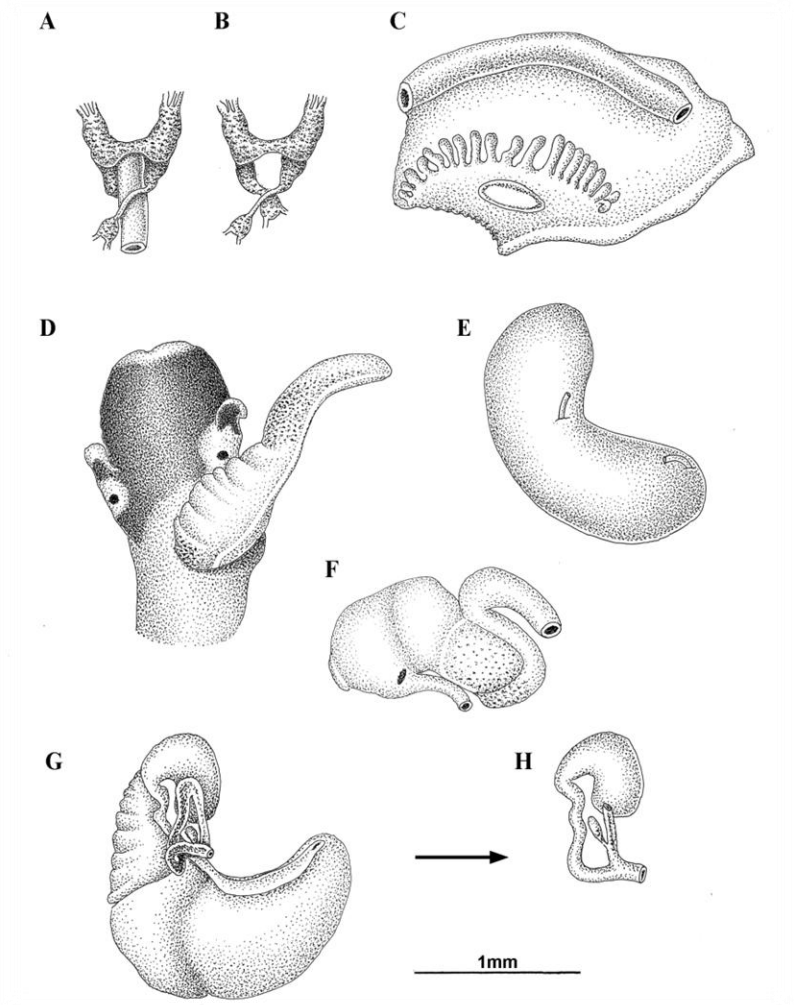


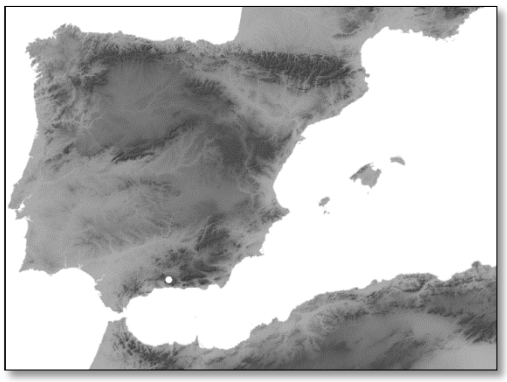
Figure 56. Anatomy of *Pseudamnicola (C.) andalusica* from type locality. **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Head of a male and penis. **E.** Prostate gland. **F.** Stomach. **G.** Female genitalia. **H.** Bursa copulatrix and seminal receptacle.

large patch of brown pigment. However, genetic divergence is clear between these two species (6.7% for COI, Appendix IV: Table 1) and anatomical differences existed to suggest this species as new (Delicado *et al.*, 2012). These differences are: (1) pallial oviduct shorter in *P. (C.) andalusica* n. sp. than in *P. (C.) luisi* and *P. (C.) marisolae*, (2) bursal duct with an expansion near the joining point with the renal oviduct in *P. (C.) marisolae* that is absent in *P. (C.) andalusica*; (3) wavy bursal duct in *P. (C.) andalusica* and straight in *P. (C.) luisi*, *P. (C.) marisolae* and *P. (C.) iruritai*; (4) longer penis in *P. (C.) andalusica* than in *P. (C.) marisolae* and *P. (C.) iruritai*, and shorter than in *P. (C.) luisi*; (5) lateral radular teeth formula 3-C-3 in *P. (C.) andalusica*, 2-C-2 in *P. (C.) luisi* and *P. (C.) marisolae* and 3/4-C-3/4 in *P. (C.) iruritai*.

***Pseudamnicola (Corrosella) ballestae* n. sp.**

Type locality

Spring and two lake in Jatar, Granada, Spain, 30S 0418451/4087781.



Type material

Holotype (ESEM preparation, Figure 57A), Paratypes (ESEM preparation, Figures 57B-E, 58, 96º ethanol and 70% ethanol, Figure 59), J.M. Barea and I. Ballesta, June 2009.

Material examined

The species has been found in a spring and two lakes near the spring in Jatar. See Appendix II.

Material Examined for Morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Tables 1-7) correspond to Lake 1, Jatar, Granada.

Etymology

Dedicated to Irene Ballesta, who nicely collected freshwater molluscs in many Andalusian localities and also material from this species.

Diagnosis

Shell with an inflated body whorl covering about 2/3 of shell length; upper region of shell aperture slightly pointed; four or five lateral cusps in central tooth of radula; intestine slightly pigmented; female genitalia with a pyriform U-shaped bursa copulatrix and elongated seminal receptacle; penis gradually tapering with a patch of black pigment in distal region; nervous system black pigmented with supraoesophageal connective about three times longer than suboesophageal.

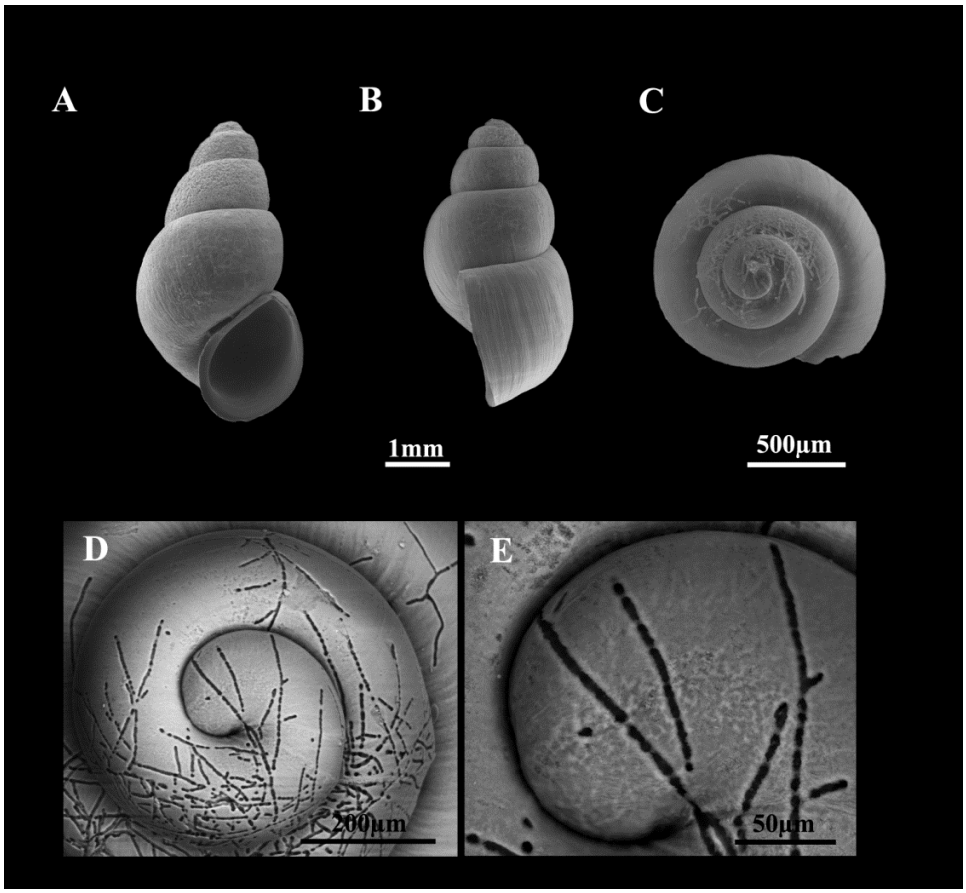


Figure 57. Shells of *Pseudamnicola (C.) ballestae* n. sp. **A-E.** Shells from Spring in Jatar, Granada. **C-E.** Protoconch and detail of protoconch microsculpture.

Description

Shell ovate-conic, yellowish with 4.25-5.5 spire whorls and between 3-5.5 mm in height (Figures 57A, B; Appendix III: Table 1); protoconch approximately 500 µm width and 1.75 whorls and a nucleus around 150 µm length (Figures 57C, D); protoconch microsculpture slightly granulated, stronger at apex (Figures 57D, F); last body whorl about 2/3 of total length; convex whorls and deep sutures; peristome frontal, complete, oval, with thicker inner lip and upper region slightly pointed; the edge of peristome is slightly sinuate (Figure 57B).

Operculum bears 1.75 spire whorls and oval muscular attachment is near nucleus (Figure 58A, B, Appendix III: Table 2).

Radula medium size (around 20% total length) and approximately ten times longer than wide (Figure 58D, Appendix III: Table 3); about 60 rows of teeth; trapezoidal central tooth with a tongue-shaped central cusp and four or five laterals, pointed tips (Figures 58D, E); lateral teeth of left column with three tapered lateral cusps and two

or three lateral cusps in the three radulae analyzed; inner marginal teeth have close to 15 pointed cusps and outer marginal teeth around 20 (Figures 58D, F).

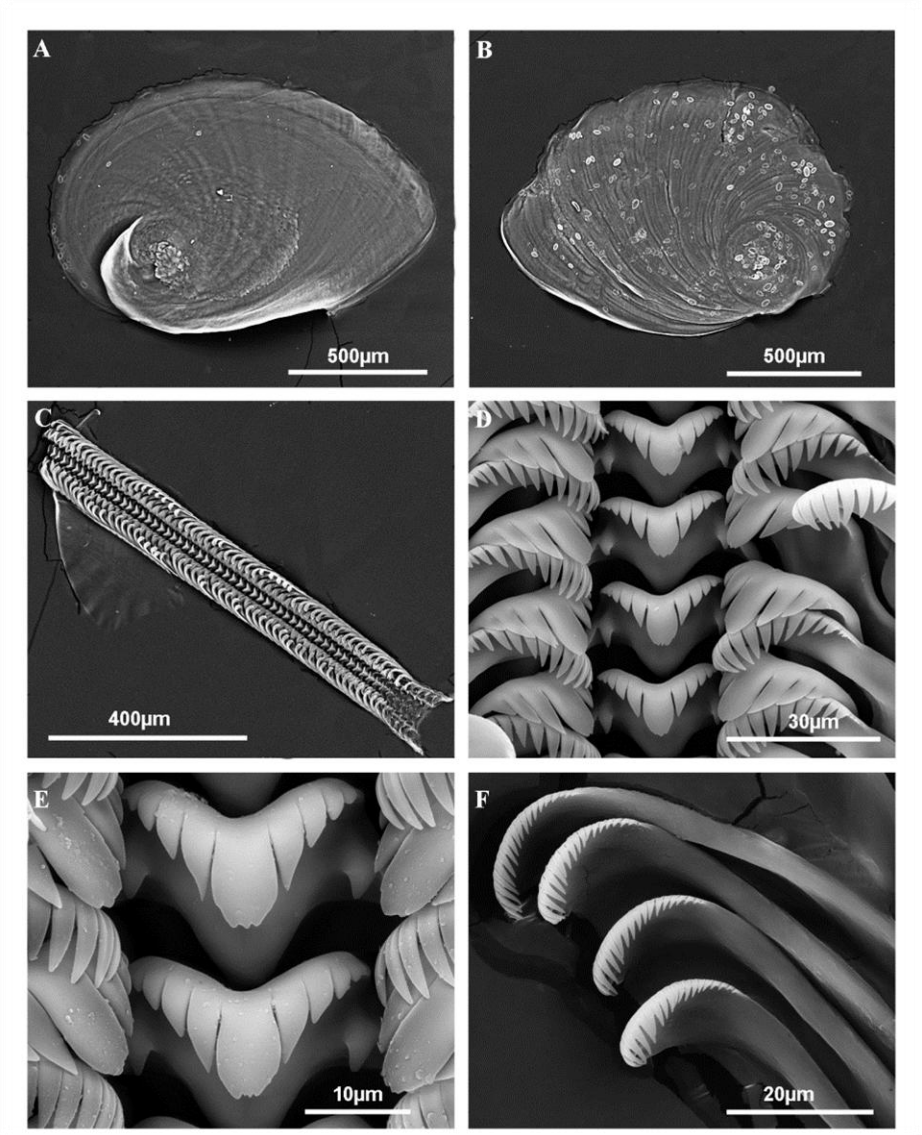


Figure 58. Operculum and radula of *Pseudamnicola (C.) ballestae* n. sp. from spring in Jatar, Granada (type locality). **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central teeth. **F.** Detail of an outer marginal tooth.

Pigmentation and anatomy. Head, dorsal region of foot and tentacles strongly brown pigmented (Figure 59F); ocular region not pigmented and tentacles with a longitudinal band without pigmentation; pigment on neck clearer than on head; snout as long as wide; foot of intermediate size. Ctenidium with 22-24 gill filaments

occupying most of pallial cavity; osphradium of intermediate width and opposite approximate middle of ctenidium (Figure 59C, Appendix III: Table 4). Stomach approximately as long as wide and style sac shorter than this one (Appendix III: Table 4); intestine has clear brown pigment (Figure 59E).

Female genitalia contains and albumen gland smaller than capsule gland (Figure 59G); bursa copulatrix pyriform U-shaped folded with straight bursal duct shorter than half bursa length (Figure 59H, Appendix III: Table 5); elongated seminal receptacle attached close to base of renal oviduct; straight renal oviduct with pale brown pigmentation from the point where it joins with the bursal duct; darker further along making several folds.

Male genitalia with a prostate gland around 2.5 times longer than wide (Figure 59D, Appendix III: Table 6); penis gradually tapering with a base of intermediate width; it contains a patch of black pigment in distal region and several folds in its midsection; it is attached behind right eye (Figure 59F); penial duct runs straight along the right side of penis.

Nervous system black pigmented, darker on ganglia than on connectives and commissures; cerebroidal ganglia equal in size as well as pleural ganglia; supraoesophageal connective about three times longer than suboesophageal (Figures 59A, B, Appendix III: Table 7); RPG ratio 0.47 in mean (moderately concentrated); straight oesophagus running beneath nervous system (Figure 59A).

Remarks. In spite of sharing more morphological characters with *Pseudamnicola (Corrosella) luisi*, *P. (C.) ballestae* n. sp. is genetically closer to *P. (C.) andalusica* (genetic divergence of 7.3%, 3.4% and 0.1% with *P. (C.) luisi* and 7%, 2.2% and 0.3% with *P. (C.) andalusica* for COI, 16S and 28S respectively). *P. (C.) ballestae* n. sp. is, together with *P. (C.) luisi* one of the longest species of this group. Some synapomorphies shared with the rest of species of *Corrosella* belonging to the same clade come to be: large conic shell (SL/SW ratio more than 1.75), pigmented and gradually tapering penis, pyriform bursa copulatrix (except cylindrical shape in *P. (C.) falkneri*) and nervous system moderately concentrated. Nevertheless, *P. (C.) ballestae* n. sp. differs from the rest of taxa belonging to the same clade in: penis shape (more tubular in *P. (C.) ballestae* n. sp.), longer seminal receptacle (0.32 mm in mean, see Appendix III: Table 5, whereas the average in the rest of species is less than 0.30 mm, except *P. (C.) luisi* which bears similar size) and the longest prostate gland (approximately 2.20 mm in mean, see Appendix III: Table 6) among *P. (Corrosella)* species.

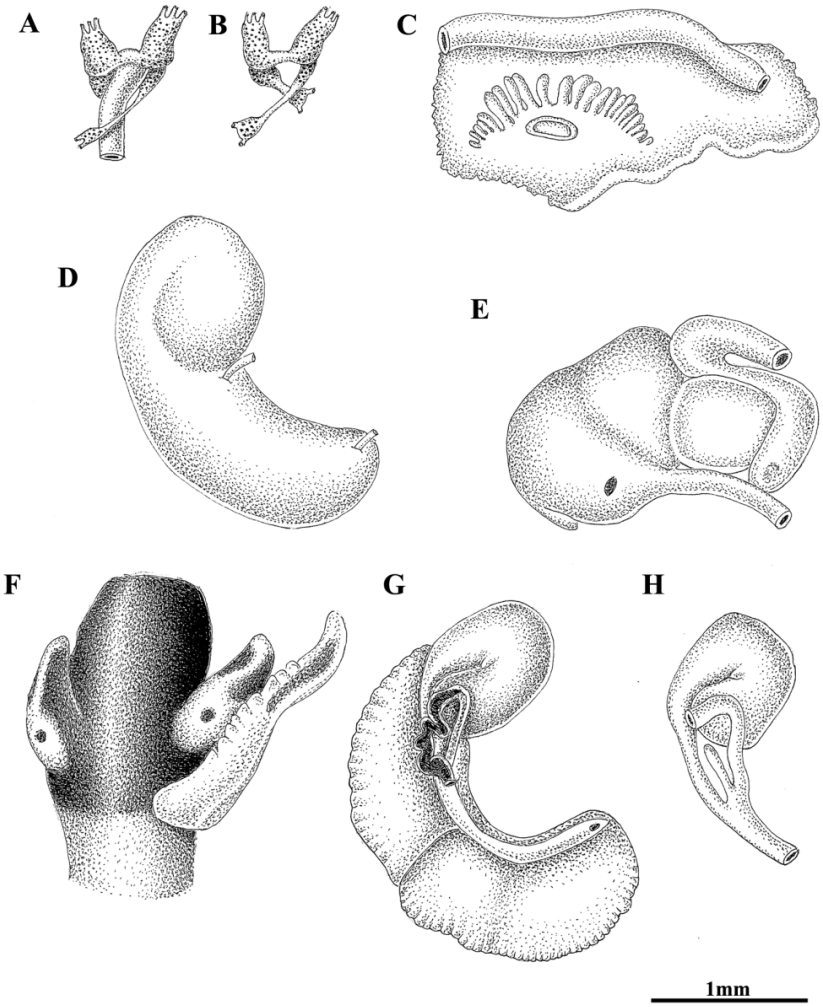
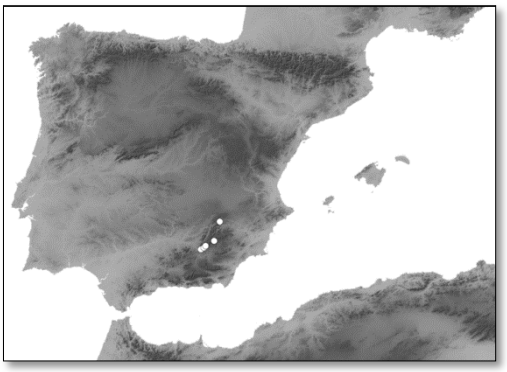


Figure 59. Anatomy of *Pseudamnicola (C.) ballestae* n. sp. from type locality. **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Prostate gland. **E.** Stomach. **F.** Head of a male and penis. **G.** Female genitalia. **H.** Bursa copulatrix and seminal receptacle.

Pseudamnicola (Corrosella) species from the North of the Iberian Peninsula are differentiated from *P. (C.) ballestae* n. sp. in: shell size (4.5 vs. less than 4 mm in height for *P. (C.) hinzi* and *P. (C.) navasiana*, see Appendix III: Table 1), bursa copulatrix shape (cylindrical in the northern species *vs.* pyriform in *P. (C.) ballestae* n. sp., Figures 46H, I, 53J-L and 59H respectively), penis shape (more triangular in species from the north), attachment of base of penis to head close to right eye in *P. (C.) ballestae* n. sp. and central in the rest of species, seminal receptacle longer in *P. (C.) ballestae* n. sp. (Appendix III: Table 5) and renal oviduct more strongly pigmented until seminal receptacle in northern species.

Pseudamnicola (Corrosella) bareai Delicado, Machordom and Ramos, 2012



Type locality

Spring at Ermita de las Santas, Collados de la Sagra, Granada, Spain, UTM: 30S 0542294/4201835.

Type material

Holotype MNCN 15.05/49190a (ESEM preparation, Figures 60A, D), paratypes MNCN 15.05/49190b (ESEM preparation, Figures 60B, D-G, 61, and 70% ethanol, Figure 62) and MNCN/ADN 34916-34930 (frozen material), D.D., 13 October 2007; JM.B., 6 June 2006, MNCN 15.05/49189 (70% ethanol); JM.B., 21 May 2008, MNCN 15.05/49191 (70% ethanol).

Other populations examined

All populations of the species were found in the Castril mountains (Granada province) with the exception on one population found at Siete Fuentes at the edge of the Cazorla Mountains in Jaén province. Specimens were collected from the localities indicated in Appendix II.

Etymology

Dedicated to José Miguel Barea, who besides being the discoverer of the type locality, kindly collaborated with us in sampling and protecting freshwater molluscs and their habitat in Andalusia.

Diagnosis

Shell with penultimate whorl tall in relation to previous ones; lateral radular tooth formula 4-C-4; intestine slightly pigmented; bursa copulatrix cylindrical U-shaped; renal oviduct darkly pigmented until insertion and seminal receptacle non-pigmented; penis gradually tapering with a clear narrow patch of pigment in the middle region; nervous system brown pigmented with supraoesophageal connective over four times longer than suboesophageal.

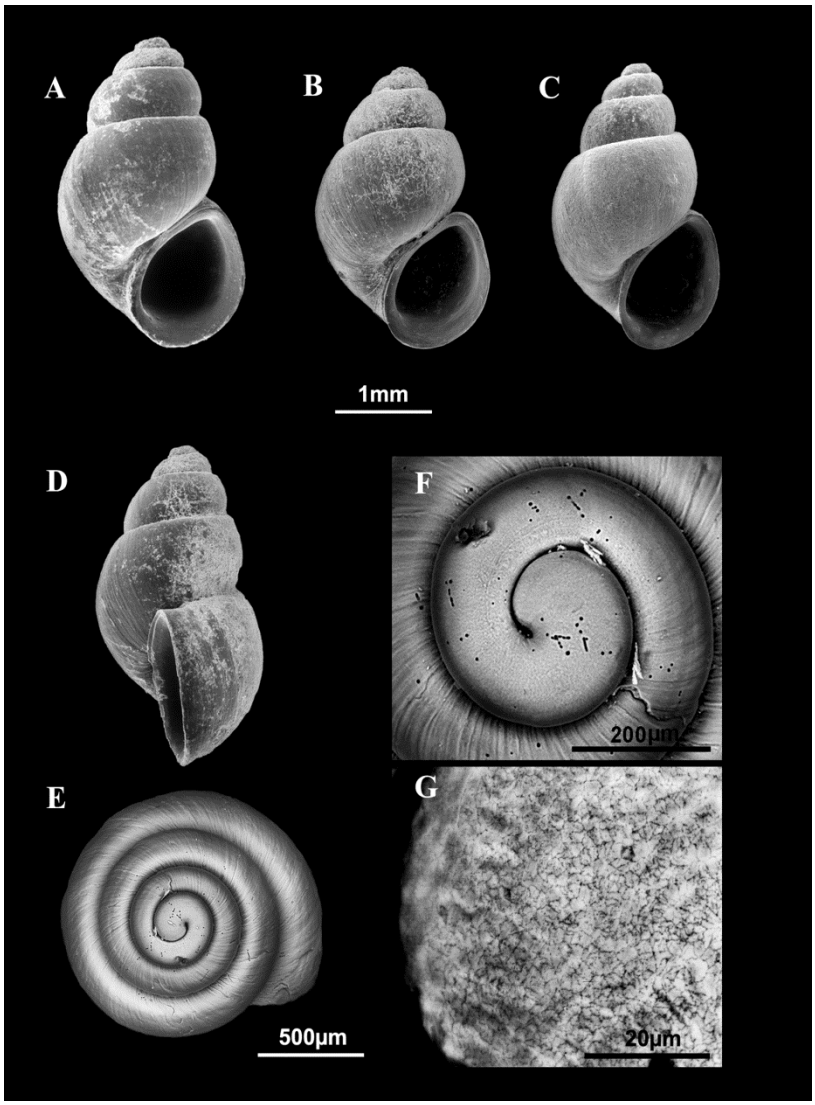


Figure 60. Shells of *Pseudamnicola (C.) bareai* **A**, **B D-G.** Shells from Spring in Ermita de las Santas, Collados de la Sagra, Granada (type locality). **C.** Shell from Agüerillo, Castril, Granada. **A, D.** Holotype (MNCN 15.05/49190a). **E-G.** Paratypes (MNCN 15.05/49190b), protoconch and detail of its microsculpture.

Description

Shell ovate-conic, yellowish periostracum, with 4-4.25 spire whorls, height 3.3-2.5 mm (Figures 60A-C, Appendix III: Table 1); body whorl occupying three-quarters of total shell length and penultimate whorl rather tall in relation to previous ones; whorls convex; protoconch net-shaped grooved (Figure 60G) with about 1.8 whorls; total width and nucleus width around 380 and 190 µm, respectively (Figures 60E, F); longitudinal ribs run parallel to protoconch suture; aperture complete oval; narrow outer lip in contact with last whorl practically hiding the umbilicus; inner lip

wider than outer; in lateral view aperture straight and slightly backwards (Figure 60D).

Operculum with around three spire whorls (Figure 61A, Appendix III: Table 2); muscle attachment area oval close to nucleus (Figure 61B).

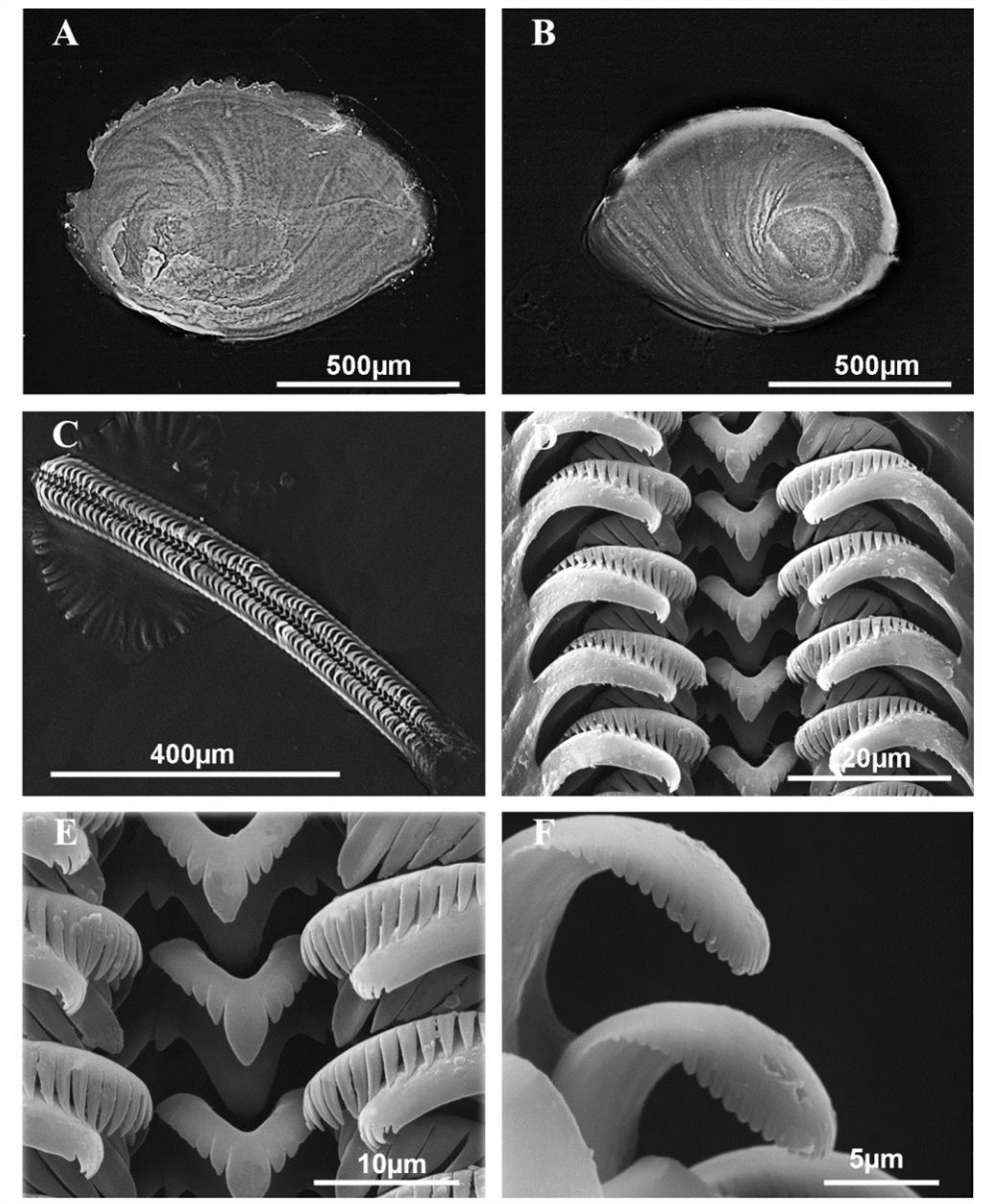


Figure 61. Operculum and radula of *Pseudamnicola (C.) bareai* from Spring at Ermita de las Santas, Collados de la Sagra, Granada (type locality). **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central tooth. **F.** Outer marginal teeth.

Radula medium size (27%) relative to maximum shell dimension and nine times longer than wide (Figure 61C, Appendix III: Table 3); around 61 rows of teeth; central tooth with a long tapered median cusp and four to six very small laterals, fused in some specimens (Figure 61D, E); basal tongue V-shaped; lateral teeth with four relatively tapered lateral cusps; inner marginal tooth contains approximately 18 cusps of decreasing size; outer marginal tooth with around 20 cusps smaller than inner marginal cusps (Figures 61D, F).

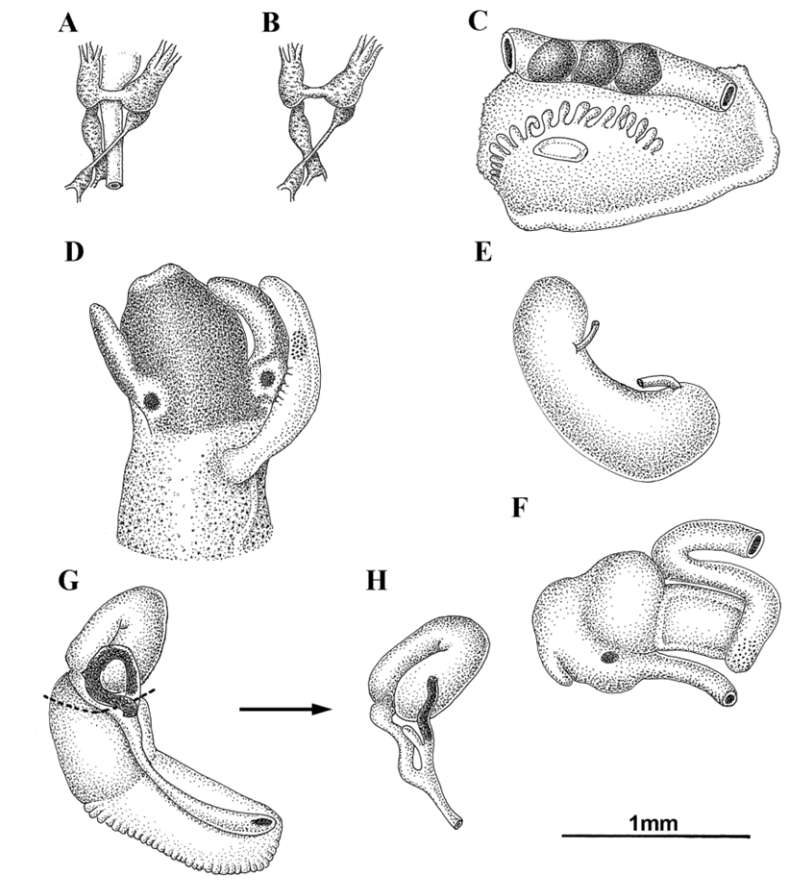


Figure 62. Anatomy of *Pseudamnicola (C.) bareai* from type locality. **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Head of a male and penis. **E.** Prostate gland. **F.** Stomach. **G.** Female genitalia. Dotted line indicates the pallial wall **H.** Bursa copulatrix and seminal receptacle.

Pigmentation and anatomy. Head with uniform blackish pigment from snout to base of penis; pigmentation clearer on neck; ocular region without pigmentation; tentacles with a longitudinal band of pigmentation on the external side (Figure 62D); snout as long as wide with medium distal lobation; foot intermediate with dorsal pigmentation. Ctenidium with 16-18 well-developed gill filaments situated in the middle of the pallial cavity; osphradium less than 50% ctenidium length and located

in the opposite middle of the ctenidium (Figure 62C, Appendix III: Table 4). Stomach slightly wider than long with two similar-size cameras and medium-sized gastric caecum (Appendix III: Table 4); style sac approximately as long as stomach; intestine weakly pigmented (Figure 62F); rectum filled with orange faecal-pellets and S-shaped in pallial cavity.

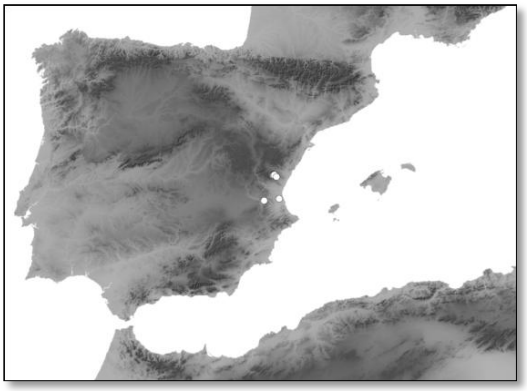
Female genitalia with an albumen gland that occupies one-third pallial oviduct (Figure 62G, Appendix III: Table 5); bursa copulatrix cylindrical U-shaped (Figure 62H) with a long duct; renal oviduct black pigmented until insertion of seminal receptacle, it makes a simple loop over the bursa copulatrix after two or three very dark loops; seminal receptacle elongated without pigment lies on renal oviduct slightly above the joining point of the bursal duct.

Male genitalia with a large bean-shaped prostate gland almost four times longer than wide and occupying a large section of pallial cavity (Figure 62E, Appendix III: Table 6); seminal duct entering the medial-posterior region of prostate gland and a pallial vas deferens emerging close to its anterior edge; penis tubular with a narrow base, six or seven small folds in the middle region and clear blackish pigmentation, varying from a small patch in the middle region to a narrow long patch covering all the middle surface (Figure 62D); penis can appear coiled in live specimens; straight penial duct running on right side of the penis from base to the tip.

Nervous system with darker ganglia than connectives and commissures; cerebral ganglia approximately same size; right pleural ganglion smaller than left pleural ganglion; supraoesophageal connective four times longer than suboesophageal (Figure 62B, Appendix III: Table 7); RPG ratio 0.42 (moderately concentrated); oesophagus runs underneath nervous system without loops or folds (Figure 62A).

Remarks. *Pseudamnicola (C.) bareai* can be distinguished from the other *P. (Corrosella)* species examined here by: lateral radular teeth with four lateral cusps on each side of the elongated central tooth, strong U-shaped bursa copulatrix and tubular penis with a narrow base and small pigment patch. *Pseudamnicola (C.) manueli* is morphologically and genetically the closest species (genetic divergence 6.7%, Appendix IV: Table 1) but both species can be differentiated because: (1) *P. (C.) bareai* has a tubular penis with a narrow base, round tip and a small patch of pigment in middle-distal region, whereas the penis in *P. (C.) manueli* is sharp with a wider base and has a longer patch of pigment in its distal portion; (2) the bursa copulatrix is folded into a U-shape in *P. (C.) bareai* and is J-shaped in *P. (C.) manueli*; (3) lateral radular tooth formula is 4-C-4 in *P. (C.) bareai* and 3-C-3 in *P. (C.) manueli*; (4) protoconch microsculpture is granular in *P. (C.) manueli* and grooved in *P. (C.) bareai*.

Pseudamnicola (Corrosella) hauffei Delicado and Ramos, 2012



Type locality

Los Nogales Spring, Benafer, Castellon, Spain, 30°55.80'N, 0°34.34' W.

Type material

Holotype MNCN 15.05/60026a (SEM preparation, Figure 63A) and paratypes MNCN 15.05/ 60026b (SEM preparation, Figures 63D-G, 64, and 70% ethanol, Figure 65) and MNCN/ADN 54952-54969 (frozen material and 70% ethanol), D.D. and C.N., 19 March 2009; MNCN 15.05/60027 (70% ethanol), 26 May 1998, B.A.

Material examined

Four males and four females from type locality were examined for anatomical study. In addition, some populations from provinces of Castellón and Valencia (Spain) were also found and studied (see Appendix II), dissecting likewise two males and two females from each for their identification.

Material examined for morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Table 1-7) were made on specimens from the type locality, Los Nogales Spring in Benafer, Castellón.

Etymology

Dedicated to the malacologist and ecologist Torsten Hauffe, for his help and support during the stay of the first author in Germany.

Diagnosis

Shell yellowish with body whorl occupying 2/3 shell length; umbilicus slightly visible; protoconch microsculpture grooved; central radular tooth formula 5-C-5; style sac protruding below non-pigmented intestine; elongate bursa copulatrix J-

shaped; renal oviduct pigmented until seminal receptacle, which has a pigmented short duct; penis triangular with a wide base attached to central area of head; nervous system brown pigmented with supraoesophageal connective about three times longer than suboesophageal.

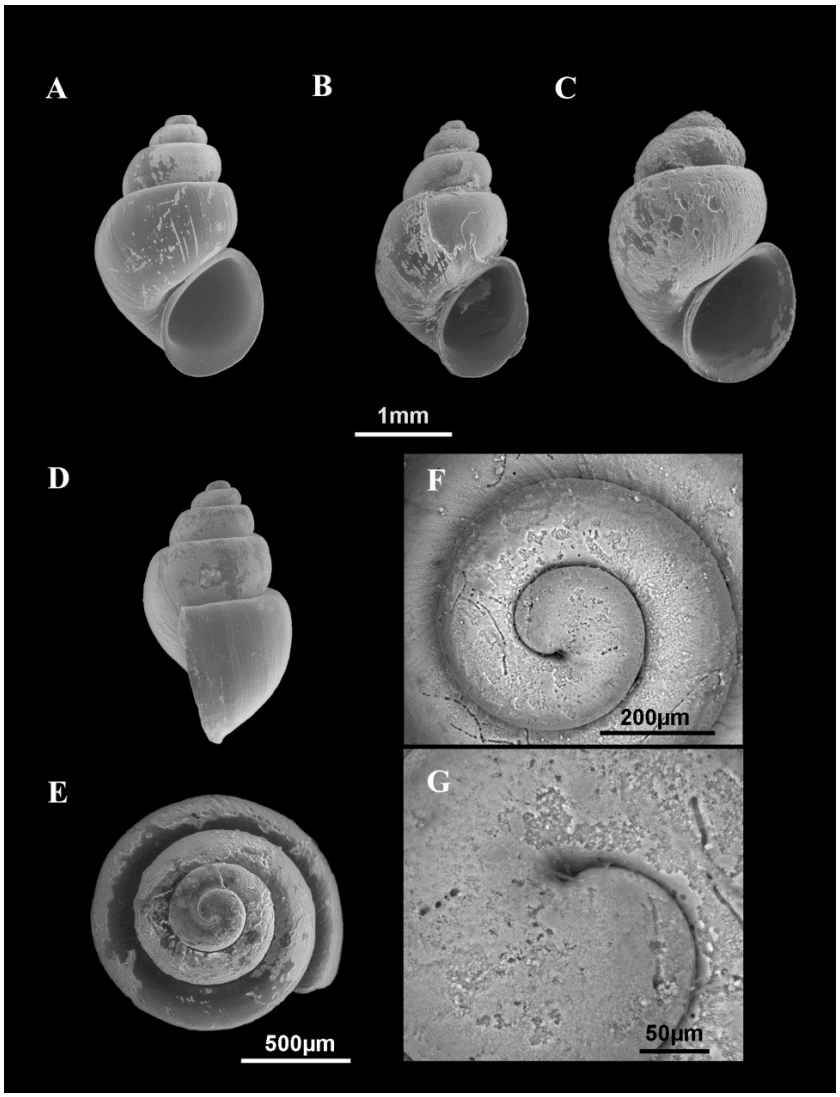


Figure 63. Shells of *Pseudamnicola (C.) hauffei*. **A**, **D-G** Shells from Nogales Spring, Benafer, Castellón, Spain. **B** Shell from San Miguel spring, Viver, Castellón, Spain. **C** Shell from Agadín spring, Benafer, Castellón, Spain. **E-G** Protoconch and microsculpture.

Description

Shell ovate-conic (Figures 63A-C), yellowish periostracum with 4-4.5 spire whorls, height around 2.0-3.0 mm (Appendix III: Table 1); protoconch approximately 450

μm wide with 1.5 whorls and a nucleus around 200 μm long (Figures 63E, F); protoconch microsculpture grooved (Figure 63G); body whorl about 2/3 total length; whorls convex with deep suture; peristome frontal, complete, oval, with thick inner lip partly hiding umbilicus; outer peristome simple, straight (Figure 63D).

Operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral, with nucleus submarginal (Figures 64A, B; Appendix III: Table 2); oval muscle attachment near nucleus.

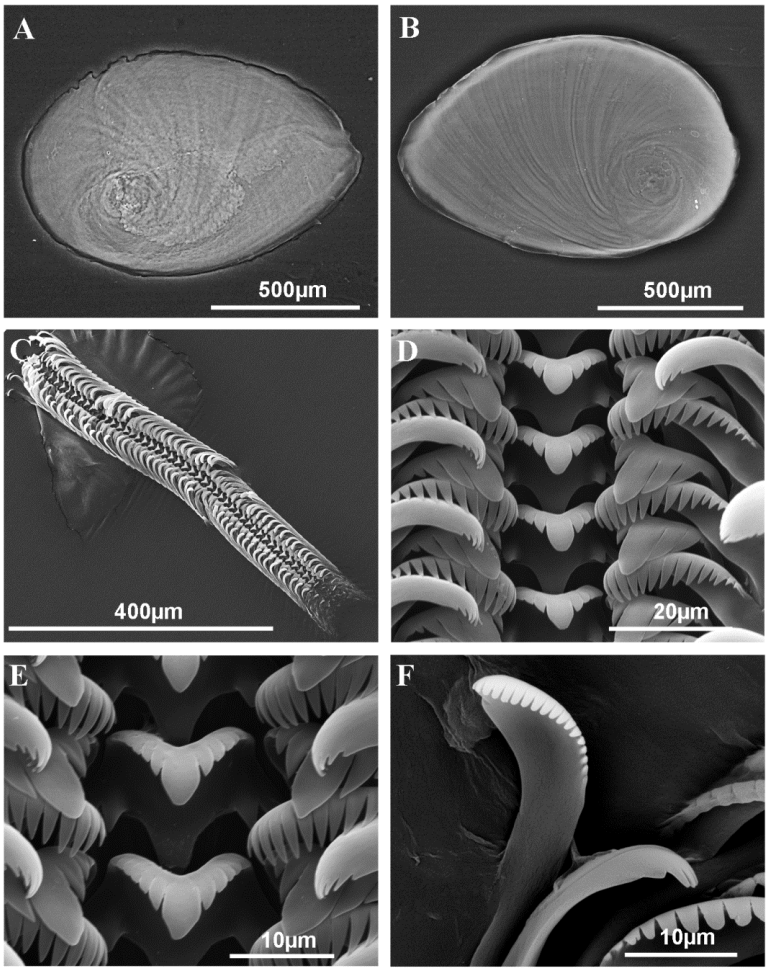


Figure 64. Operculum and radula of *Pseudamnicola (C.) hauffei* from Nogales Spring, Benafer, Castellón, Spain. **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central tooth. **F.** External marginal teeth.

Radula with around 50 rows of teeth, medium in size (25% total shell length) (Figure 64C, Appendix III: Table 3); central tooth with a tongue-shaped median

cusp and five lateral cusps, slightly sharpening towards central one (Figures 64D, E); lateral teeth with a long tongue-shaped median cusp and three tapered laterals; inner and outer marginal teeth bear 15 and 19 sharp cusps respectively (Figures 64D, F).

Pigmentation and anatomy: Head intensely brown pigmented from snout to neck (Figure 65D); pigment on neck clearer than on head; brown band of pigment also on tentacles, but not on ocular lobes; snout as long as wide, with medial lobation; foot intermediate length, pigmented on dorsal region. Ctenidium in the anterior region of pallial cavity with about 15 gill filaments; osphradium ellipsoidal under central gill filaments (Figure 65C, Appendix III: Table 4). Stomach slightly longer than wide (Figure 65F); style sac barely shorter than stomach, protruding below intestine (Appendix III: Table 4).

Female genitalia with a pallial oviduct about four times longer than wide (Figure 65G; Appendix III: Table 5); capsule gland slightly longer than albumen gland and denser in posterior region; genital aperture in the anterior extreme of pallial oviduct; elongate bursa copulatrix, J-shaped folded with a duct less than 50% bursa length; renal oviduct scarcely pigmented from the insertion point of bursal duct to where it begins to fold and black pigmented, making two or three loops; elongate seminal receptacle with pigmented short duct (Figure 65H) joining renal oviduct slightly above the point where the bursal duct joins the renal oviduct.

Male genitalia bearing a bean-like prostate gland about three times longer than wide (Figure 65E, Appendix III: Table 6); penis triangular with a wide base attached to central area of head with some folds in middle section and a narrow patch of black pigment on distal surface (Figure 65D); vas deferens uncoiled in penis running straight close to the external margin.

Nervous system brown pigmented, but ganglia darker than connectives and commissures; cerebral ganglia equal in size; supraoesophageal and suboesophageal ganglia similar in shape and size; supraoesophageal connective around three times longer than suboesophageal (Figures 65A, B, Appendix III: Table 7). Mean RPG ratio 0.51 (elongate).

Remarks. Some of the localities where this species was found were cited by Gasull (1981) incorrectly as inhabited by *P. (C.) astieri*. Both species show marked differences such as: 1) *P. (C.) astieri* has a longer shell, longer spire (SL-LBW) (Appendix III: Table 1) and the protoconch microsculpture is more granulated than in *P. (C.) hauffei*. Moreover, the inner lip of the shell aperture in *P.* (C.) *hauffei* is thicker than in *P. (C.) astieri* and partly hides the umbilicus; 2) central radular tooth with seven lateral cusps in *P. (C.) astieri*, five in *P. (C.) hauffei* (Table 4); 3) style sac surrounded by black pigmented intestine in *P. (C.) astieri* yet lacks pigment and protrudes under the intestine in *P. (C.) hauffei* (Figures 39F and 65F); 4) bursa

copulatrix J-shaped and seminal receptacle with a short duct in *P. (C.) hauffei*, while bursa copulatrix is U-shaped and seminal receptacle is shorter and lacks a duct in *P. (C.) astieri* (Figures 39H and 65H)*;* 5) penis triangular with a wide base in *P. (C.) hauffei* and slender in *P. (C.) astieri* (Figures 39D and 65D); 6) nervous system elongate (RPG= 0.51) in *P. (C.) hauffei* yet moderately concentrated (RPG = 0.42) in *P. (C.) astieri.*

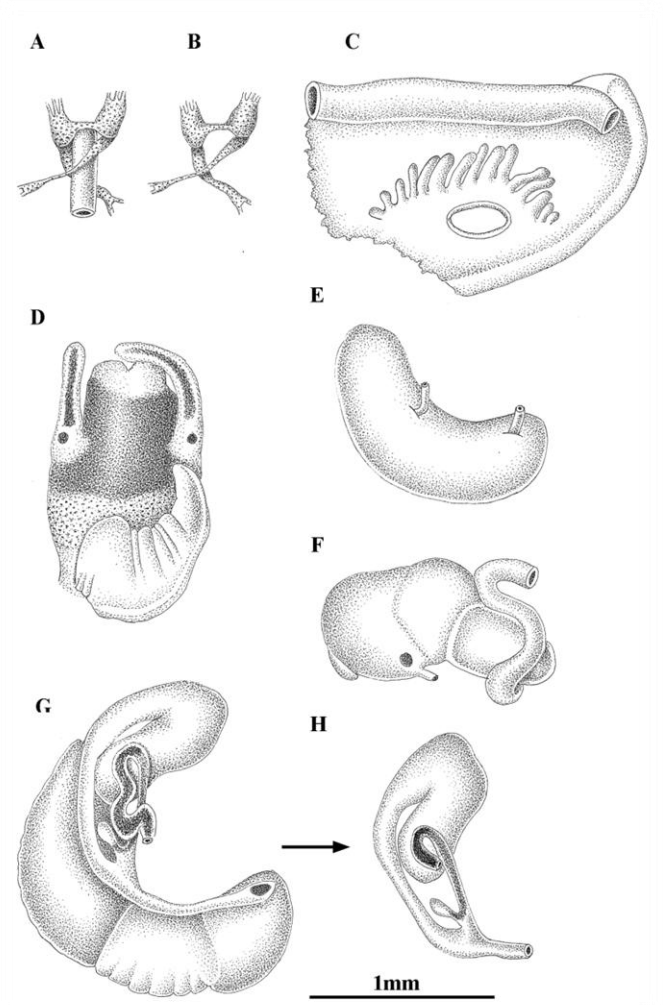
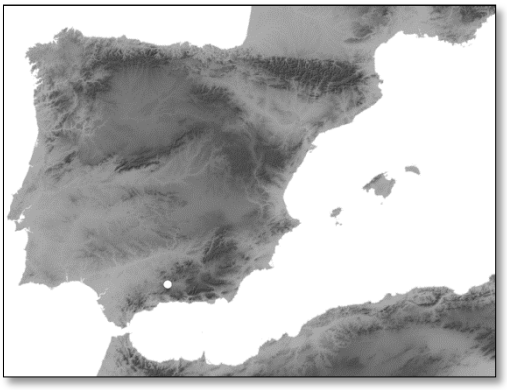


Figure 65. Anatomy of *Pseudamnicola (C.) hauffei* from Nogales Spring, Benafer, Castellón, Spain. **A, B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Head of a male and penis. **E.** Prostate gland. **F.** Stomach. **G.** Female genitalia. **H.** Bursa copulatrix and seminal receptacle.

Compared to the other *P.* (*Corrosella*) species living in nearby areas, *P. (C.) hinzi* and *P. (C.) navasiana, P. (C.) hauffei* has a shorter and more ovate shell shape, a longer bursa copulatrix, bursa duct and seminal receptacle, and a more triangular wider-based penis.

Pseudamnicola (Corrosella) iruritai Delicado, Machordom and Ramos, 2012



Type locality

Don Pedro Spring at Loja, Granada, Spain, UTM: 30S 0399460/4115074.

Type material

One shell was selected as holotype, that, although eroded, represents the most frequent shape of the shells of *P. (C.) iruritai*. As a result of extensive erosion of this species' shells, paratypes are very important to provide a reference model of the species. Therefore, the type material consists of: holotype MNCN 15.05/53708a (ESEM preparation, Figure 66B). Paratypes of around 80 specimens collected at different times: JM.B., MNCN 15.05/53708b (96% ethanol) and MNCN/ADN 34967-34969 (96% ethanol); JM.B., MNCN 15.05/53709 (96% ethanol); D.D. and C.N., 20 April 2009, MNCN 15.05/53710 (70% ethanol, absolute ethanol and ESEM preparation, Figures 66, 67, 68) and MNCN/ADN 34970-34974 (absolute ethanol); I.B., 16 February 2010, MNCN 15.05/53711 (70% ethanol) and MNCN/ADN 34975-34976, 39780 (absolute ethanol).

Other populations examined

This species has been found at only one further locality near the type locality: ditch of Don Pedro Spring, Loja, Granada, UTM: 30S 0399460/4115074, D.D. and C.N., 19 April 2009, MNCN 15.05/53712 (70% ethanol) and MNCN/ADN 34947-34951 (96% ethanol).

Etymology

Dedicated to José María Irurita, Head of the Flora and Fauna Department (Delegación Provincial de Granada, Consejería de Medio Ambiente, Junta de Andalucía) for his contribution to the knowledge of freshwater hydrobiid fauna and invertebrate conservation in Andalusia.

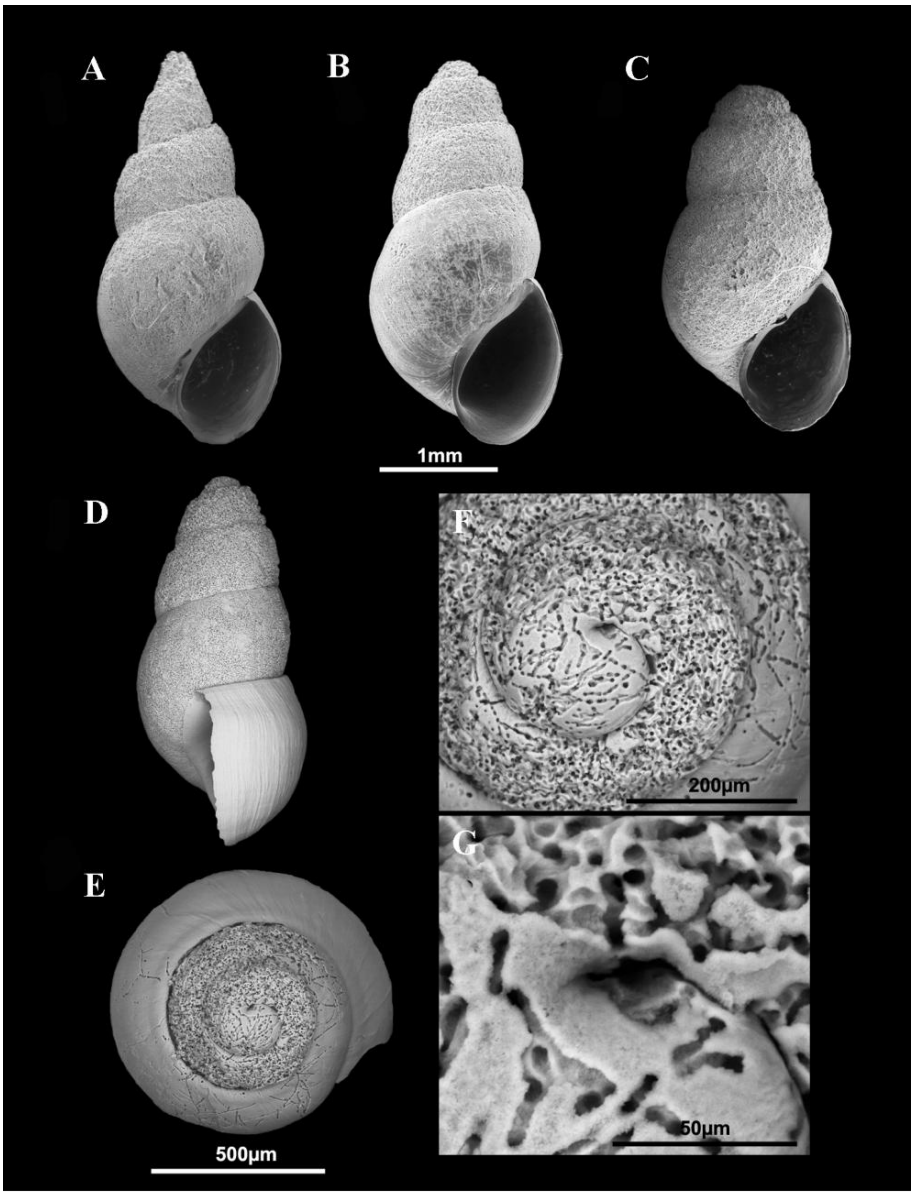


Figure 66. Shells of *Pseudamnicola (C.) iruritai*. **A-G.** Shells from Don Pedro Spring in Loja, Granada (type locality); **B.** Holotype. **E-G.** Protoconch very eroded.

Diagnosis

Shell slender, inner lip rather thicker than outer; body whorl surface striated; pigmented intestine; female genitalia with a pyriform non-folded bursa copulatrix and a globular seminal receptacle with short duct; penis tapered with a small patch of black pigment in distal region, folded in the middle area; nervous system brown pigmented with a supraoesophageal connective around three times longer than suboesophageal.

Description

Shell yellowish periostracum with 4.5-5.5 spire whorls, height 4.20-3.30 mm (Figures 66A-C, Appendix III: Table 1); body whorl occupies half of shell length, surface striped; teleoconch and protoconch usually very eroded, being difficult to assess the number of spire whorls; protoconch around 310 μm wide and nucleus width 140 μm (Figures 66E, F); protoconch microsculpture non-appreciable even in juveniles because of surface grooves and folds due to erosion (Figure 66G); peristome frontal, oval, complete, with thin outer lip and thicker inner lip in contact with body whorl hiding umbilicus; outer peristome simple, straight and very fragile (Figure 66D).

Operculum with around 3.5 spire whorls (Figure 67B, Appendix III: Table 2); internal side has a convex edge and oval muscular attachment is near nucleus (Figure 67A).

Radula medium size (22% total shell length) and approximately seven times longer than wide (Figure 67C, Appendix III: Table 3); around 50 rows of teeth; trapezoidal central tooth with a tongue-shaped central cusp and six laterals of decreasing size, pointed tips (Figures 67D, E); lateral teeth of left column with four tapered lateral cusps next to central one and three lateral cusps in right column in the three radulae analyzed; inner marginal tooth has approximately 20 pointed cusps and outer marginal tooth around 25 shorter cusps (Figures 67D, F).

Pigmentation and anatomy. Head and dorsal side of tentacles dark brown pigmented (Figure 68D); ocular region not pigmented; dorsal region of foot also pigmented; pigment on neck clearer than on head; snout as long as wide and tentacles longer than snout; foot of intermediate size. Ctenidium with around 18 well-developed gill filaments occupying most of pallial cavity; osphradium appears in opposite middle of the ctenidium and is two times longer than wide (Figure 68C, Appendix III: Table 4). Stomach with a posterior chamber larger than anterior, caecum relatively long (Appendix III: Table 4); style sac shorter than stomach projected under intestine in some specimens; intestine has clear brown pigment (Figure 68F); rectum slightly S-shaped in pallial cavity.

Female genitalia contains an albumen gland smaller than capsule gland with two regions (anterior region is more whitish) (Figure 68G); bursa copulatrix pyriform non folded with straight bursal duct shorter than bursa length (Figure 68H, Appendix III: Table 5); pyriform seminal receptacle attached close to base of renal oviduct; straight renal oviduct with pale brown pigmentation from the point where it joins with the bursal duct; darker further along making one or two folds.

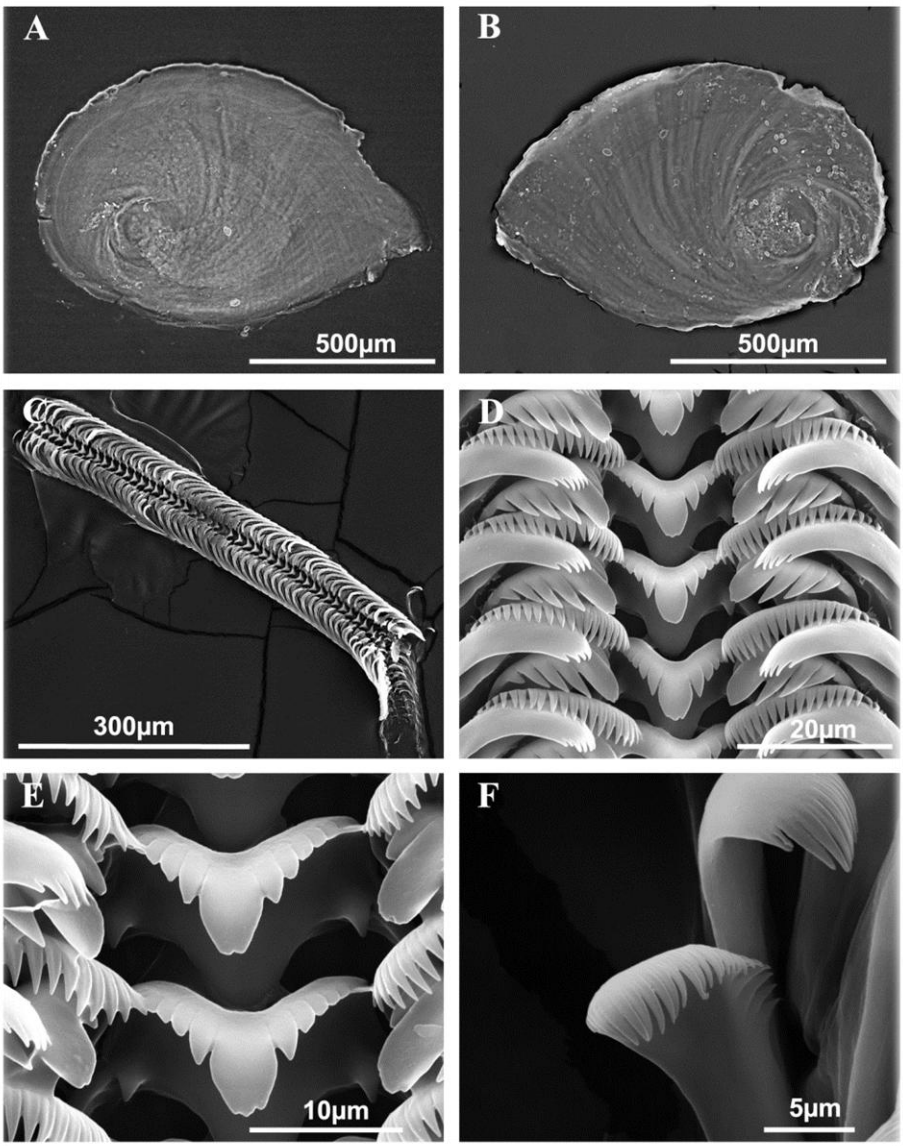


Figure 67. Operculum and radula of *Pseudamnicola (C.) iruritai* from Don Pedro Spring in Loja, Granada (type locality). **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central teeth. **F.** Detail of outer marginal teeth.

Male genitalia with a prostate gland three times longer than wide (Appendix III: Table 6); vas efferens entering the medial-posterior region and pallial vas deferens emerging from the anterior region (Figure 68E); penis pointed with a wide base, some folds in the middle section and a small expansion near the tip where it contains a patch of clear brown pigment; it is attached to the central region of head (Figure 20D); penial duct runs straight along the right side of penis.

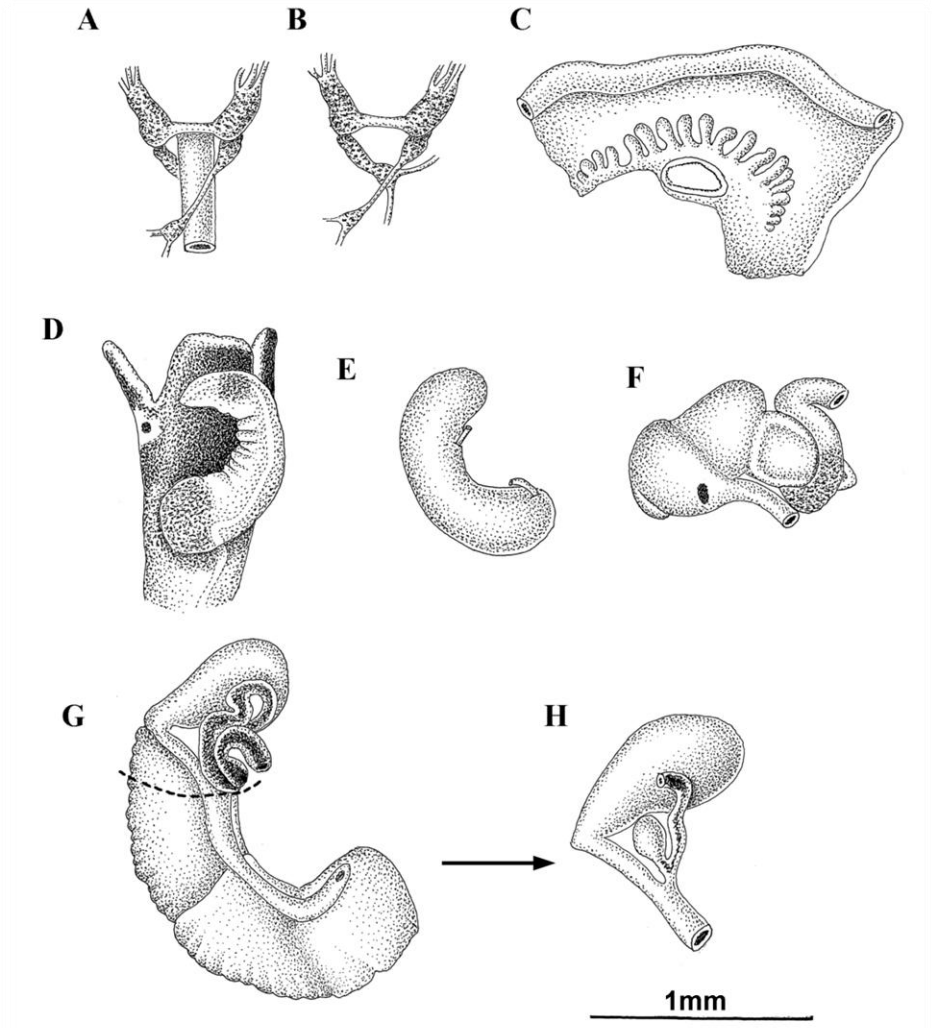


Figure 68. Anatomy of *Pseudamnicola (C.) iruritai* from type locality. **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Head of a male and penis. **E.** Prostate gland. **F.** Stomach. **G.** Female genitalia. **H.** Bursa copulatrix and seminal receptacle.

Nervous system brown pigmented, darker on ganglia than on connectives and commissures; cerebroidal ganglia equal in size as well as pleural ganglia; supraoesophageal connective more than three times longer than suboesophageal (Figure 68B, Appendix III: Table 6); RPG ratio 0.44 (moderately concentrated); straight oesophagus running beneath nervous system (Figure 68A).

Remarks. This species was only found at two very close sites in the Granada province, intra- and inter-population variability being scarce. More variable quantitative characters are: width of penis base, length of bursa copulatrix and seminal receptacle, and length of nervous connectives and commissures. Another variable character is the position of the intestine with respect to the style sac, which

either surrounds the sac or crosses it. This character varies among specimens even within populations.

Pseudamnicola (C.) iruritai is the only species of the *Corrosella* subgenus with an extensively eroded shell surface, a pyriform bursa copulatrix and asymmetry in radular columns of lateral teeth (four lateral cusps on left column and three on right column in the three radulae studied).

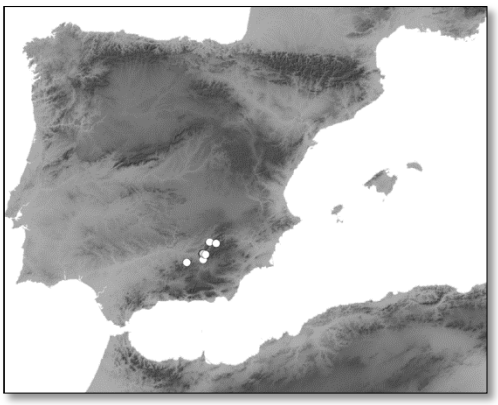
In addition to a different shape of the bursa copulatrix, the seminal receptacle is globose and larger than in the rest of the species, especially compared with *P. (C.) falkneri* and *P. (C.) bareai*, whose seminal receptacles are half the size of those in *P. (C.) iruritai*. The penis is pointed as in *P. (C.) manueli*, but wider, longer and the distal pigment patch is rounder and paler in *P. (C.) iruritai*. This species also shows slight pigmentation at the base of the penis.

Pseudamnicola (C.) iruritai belongs to the same clade as *P. (C.) luisi*, *P. (C.) marisolae* and *P. (C.) andalusica* (Figure 26), showing similar shell and penis morphology. However *P. (C.) iruritai* differs from these according to the following features: (1) the size of specimens, being the smallest in the clade; (2) six lateral cusps on the central radular tooth in *P. (C.) iruritai*, whereas in *P. (C.) marisolae* and *P. (C.) andalusica*, the number of cusps is four and in *P. (C.) luisi* it is three; (3) 20 cusps on inner marginal radular teeth (11, 12 and 13 in *P. (C.) marisolae*, *P. (C.) luisi* and *P. (C.) andalusica*, respectively); (4) bursal duct much shorter than in the other three species (Appendix III: Table 5); (5) the bursa copulatrix non-folded yet folded in the others; (6) the seminal receptacle in *P. (C.) iruritai* is globular yet elongated in the rest.

Pseudamnicola (Corrosella) manueli Delicado, Machordom and Ramos, 2012

Type locality

La Garganta Stream in Nava de San Pedro, Jaén, Spain, UTM: 30S 0509318/ 4194330.



Type material

Holotype MNCN 15.05/49176a (SEM preparation, Figures 69A, D) and paratypes MNCN 15.05/49176b (SEM preparation, Figures 69E-G,

70, and 70% ethanol, Figure 71) and MNCN/ADN 34899-34904 (frozen material), D.D., 12 October 2007; MNCN 15.05/49175 (70% ethanol), 1 May 1990, D.M.

Other populations studied

In addition to type specimens, we examined the following material collected in the province of Jaén (see Appendix II).

Specimens examined for morphometry

Shell, anatomical, operculum and radular measurements (Appendix III: Tables 1-7) were made in male and female specimens collected at La Garganta stream in Nava de San Pedro (type locality), Jaén in March, April, May and October.

Etymology

Dedicated to Manuel Delicado, father of the author, for his support and help with fieldwork.

Diagnosis

Shell with a bulging inflated body whorl relatively wider than the rest of whorls; oesophagus and intestine without pigmentation; anterior edge of style sac protrudes under the intestine; female genitalia with an elongated pyriform J-shaped bursa copulatrix, seminal receptacle slightly pigmented, above insertion of bursal duct; renal oviduct black pigmented until insertion point of seminal receptaculum; penis gradually tapering and pointed at its end, with a narrow distal pigment patch; nervous system black pigmented with supraoesophageal connective over four times longer than suboesophageal.

Description

Shell ovate-conic, yellowish periostracum with 4.25-4.75 spire whorls, height 3.7-3 mm (Figures 69A-C; Appendix III: Table 1); body whorl well-developed, about three quarters of shell length, and wider than the rest of whorls; deep suture and convex spire whorls; protoconch with approximately 1.8 whorls; protoconch width and width of nucleus around 420 μm and 180 μm, respectively (Figures 69E, F); protoconch microsculpture granulated (Figure 69G); oval aperture complete with thin outer lip and thicker inner lip; narrow umbilicus; edge of peristome straight (Figure 69D).

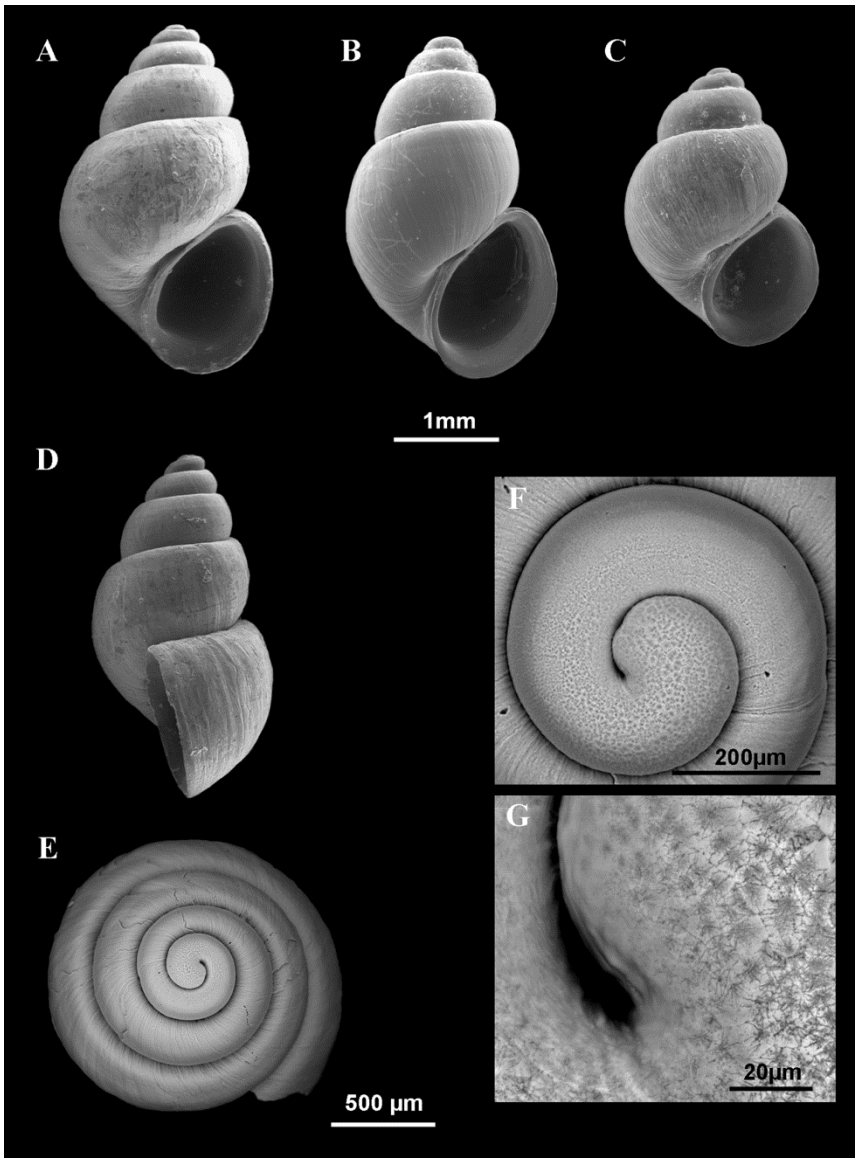


Figure 69. Shells of *Pseudamnicola (C.) manueli*. **A**, **D-G.** Shells from La Garganta stream in Nava de San Pedro, Jaén (type locality). **B.** Shell from Arroyo del Valle, La Iruela, Jaén. **C.** Shell from El Céfano spring in La Iruela, Jaén. **A**, **D.** Holotype. **E-G.** Paratypes: protoconch and microsculpture from the protoconch.

Operculum with around 2.5 spire whorls and an oval muscle attachment area near the nucleus (Figures 70A, B, Appendix III: Table 2).

Radula medium size (18%) relative to maximum shell dimension (Figure 70C, Appendix III: Table 3); with approximately 50 rows of teeth; central tooth with a large median cusp, sometimes slightly divided, and four or five lateral very small cusps of irregular shape decreasing in size (Figures 70D, E) giving the edge a

serrated appearance; lateral teeth with three sharp lateral cusps; inner marginal teeth with approximately 18 tapered cusps and outer marginal teeth with around 22 tapered cusps smaller than inner marginal cusps (Figures 70D, F).

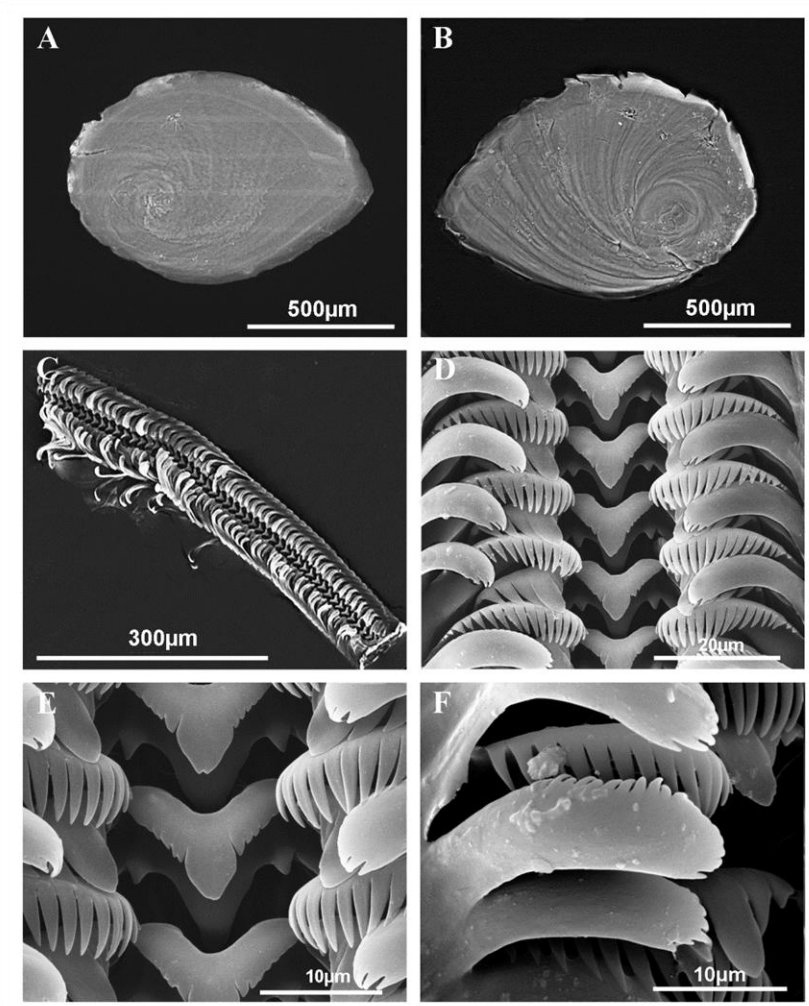


Figure 70. Operculum and radula of *Pseudamnicola (C.) manueli* from La Garganta Stream in Nava de San Pedro, Jaén (type locality). **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Detail of central tooth. **F.** Inner and outer marginal teeth.

Pigmentation and anatomy. Head dark brown pigmented; internal side of the tentacle has a longitudinal streak without pigmentation (Figure 71D); foot intermediate in size and with dark brown pigment on its dorsal side. Ctenidium well-developed with 17-19 gill filaments taller than wide occupying two-thirds of pallial cavity; osphradium in opposite middle region of ctenidium (Figure 71C, Appendix III: Table 4). Stomach almost as long as wide with both chambers equal in size

(Appendix III: Tables 4); style sac with protruding intestinal loop; oesophagus and intestine without pigmentation (Figure 71F); rectum S-shaped in pallial cavity containing orange faecal pellets.

Female genitalia with two glands in pallial oviduct: albumen gland, the most posterior, and capsule gland with two portions (the most proximal is whiter); albumen gland occupies more than one-third of pallial oviduct (Figure 71G, Appendix III: Table 5); bursa copulatrix elongated J-shaped (Figure 71H); black pigmented renal oviduct fading from loop to insertion of seminal receptacle; renal oviduct lies over bursa copulatrix making two or three loops; elongated seminal receptacle without duct, situated on renal oviduct above the insertion of bursal duct.

Male genitalia with a bean-shaped prostate gland (Figure 71E, Appendix III: Table 6) the seminal duct entering the posterior region and a pallial vas deferens emerging close to its anterior edge; penis simple, gradually tapered, with a distal patch of pigmentation and six or seven folds in its middle region (Figure 71D); the penis is attached to the central region of head, behind the eyes; penial duct straight or slightly undulating on the right/external side of the penis, running from base to penis tip.

Nervous system black pigmented, darker on ganglia than connectives and commissures; cerebral ganglia approximately same size; supraoesophageal connective is four times longer than suboesophageal connective (Figures 71A, B, Appendix III: Table 7); RPG ratio is 0.44 (moderately concentrated); oesophagus running straight beneath cerebral commissure.

Remarks. Populations from Nava de San Pedro (type locality), la Mata and Padros de la Presa have shells with a wider body whorl and a more tapered penis with a small patch of pigmentation, whereas the shells of specimens from La Iruela and Cazorla village are more slender and the penis has a rounded tip and larger pigment patch. Furthermore, the pigmented area on the seminal receptacle is larger in females of the type locality than the rest. Molecular analyses also indicate certain genetic distance between the two groups of populations (3.9% for COI) (Appendix IV: Table 1), but this distance is insufficient to consider them two different species.

Pseudamnicola (C.) manueli differs from the rest of the species of *Corrosella* examined in having: an inflated body whorl of shells (Figures 69A-C), granulated protoconch microsculpture (Figure 69G), presence of four or five lateral very small cusps of the central radula tooth giving a serrated appearance (Figures 70D, E, Appendix III: Table 3), long ctenidium (the longest being a mean of 1.43 mm, Appendix III: Table 4) and a tapered penis.

Pseudamnicola (C.) bareai is the genetically closest species to *P. (C.) manueli* (5.46% divergence) and both share characters such as: shell dimensions

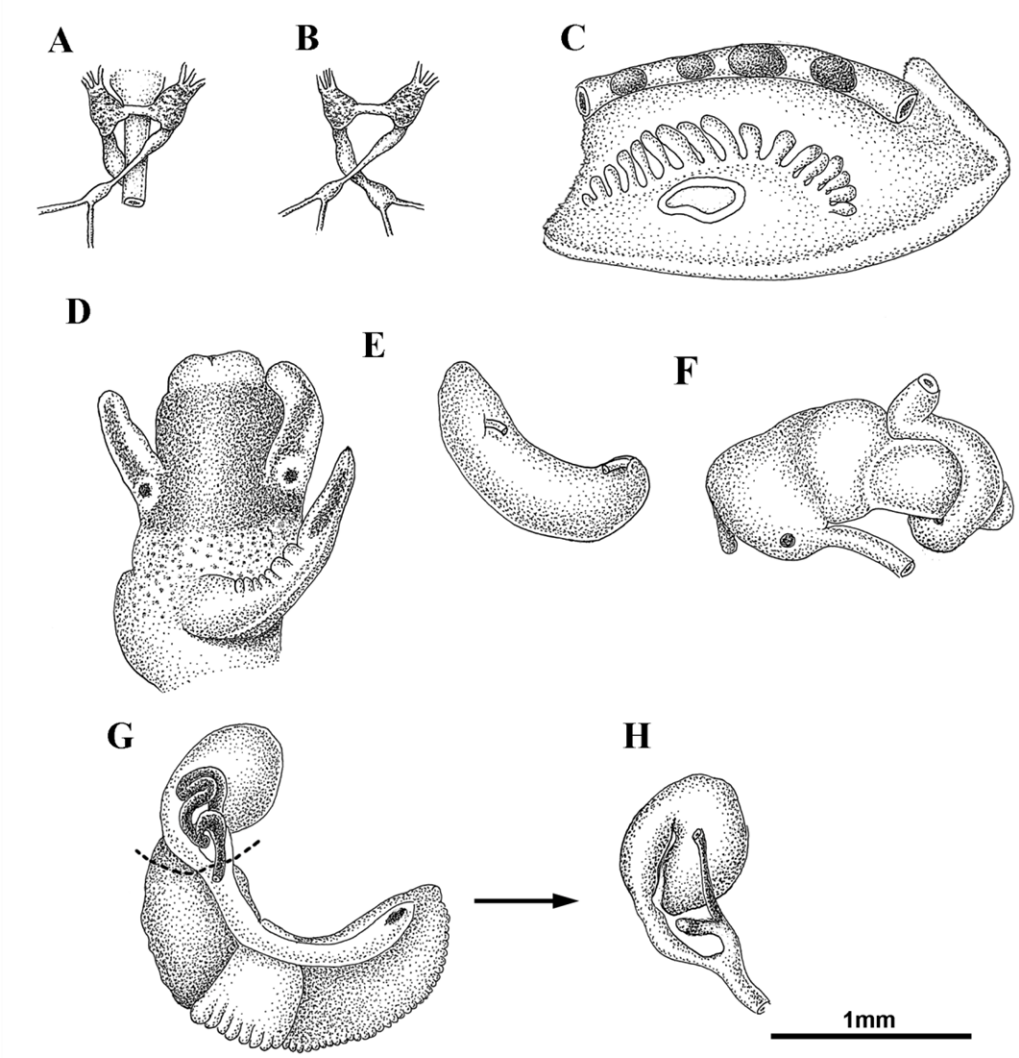
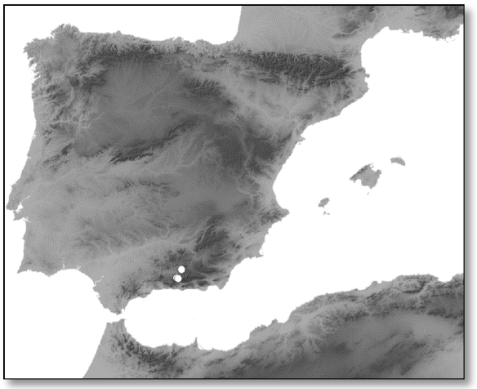


Figure 71. Anatomy of *Pseudamnicola (C.) manueli* from type locality. **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Head of a male and penis. **E.** Prostate gland. **F.** Stomach. **G.** Female genitalia. Dotted line shows the pallial wall. **H.** Bursa copulatrix and seminal receptacle.

(Appendix III: Table 1), 18 cusps in inner marginal teeth, length of penis (1 mm approximately in both species) and prostate gland (1.6 mm average length). However, there are some characters that serve to differentiate both species: (1) central radular tooth with a wide, almost pentagonal, central cusp in *P. (C.) manueli*, being narrower and more elongated in *P. (C.) bareai*; (2) three lateral cusps in lateral radular teeth in *P. (C.) manueli* and four in *P. (C.) bareai*; (3) different appearance and size of capsule gland (Figure 71G; Appendix III: Table 5), being longer in *P. (C.) manueli* and showing two regions of different opacity, whereas in *P. (C.) bareai*

its appearance is more uniform; (4) elongated bursa copulatrix folded into a J-shape in *P. (C.) manueli* without clear transition from bursal duct, which widens gradually (Figure 71H), and folded into a U-shape in *P. (C.) bareai* (Figure 62H) with clear transition from the bursal duct; (5) sharp penis with a long patch of pigmentation in distal region in *P. (C.) manueli* (Figure 71D), and round penis tip and smaller almost round patch of pigment in middle region of penis in *P. (C.) bareai* (Figure 62D).

Pseudamnicola (Corrosella) marisolae Delicado, Machordom and Ramos, 2012



Type locality

Pilar del Mono Spring in Dúrcal, Granada, Spain, UTM: 30S 0449218/4095030.

Type material

Holotype MNCN 15.05/49417a (ESEM preparation, Figure 72A), paratypes MNCN 15.05/49417b (ESEM preparation, Figures 72D-G, 73, 70% ethanol, Figure 74, D.M and J.T., 17 October 1989; D.M. and E.R., 25 September 1989, MNCN 15.05/49416 (70% ethanol); D.M., 15 October 1990, MNCN 15.05/49418 (70% ethanol); D.M., 8 February 1992, MNCN 15.05/49419 (70% ethanol); B.A., 27 March 1998, MNCN15.05/49420 (70% ethanol); JM.B., 22 September 2008, MNCN 15.05/49421 (96% ethanol) and MNCN/ADN 34945-34946 (96% ethanol).

Other populations examined

Specimens of this species were found in the centre and southern area of Granada (see Appendix II).

Etymology

Dedicated to María Soledad Iglesias (Marisol), mother of the author, for her help in collecting the material and her constant support.

Diagnosis

Shell slender of marked conic shape; central radular tooth with four lateral cusps; intestine and oesophagus without pigmentation; renal oviduct brown or black pigmented; bursal duct long and narrow except at the point joining renal oviduct where it has an expansion; penis long with a large patch of black pigment from middle region to tip and folds on the base and middle section; nervous system with a supraoesophageal connective around four times longer than suboesophageal.

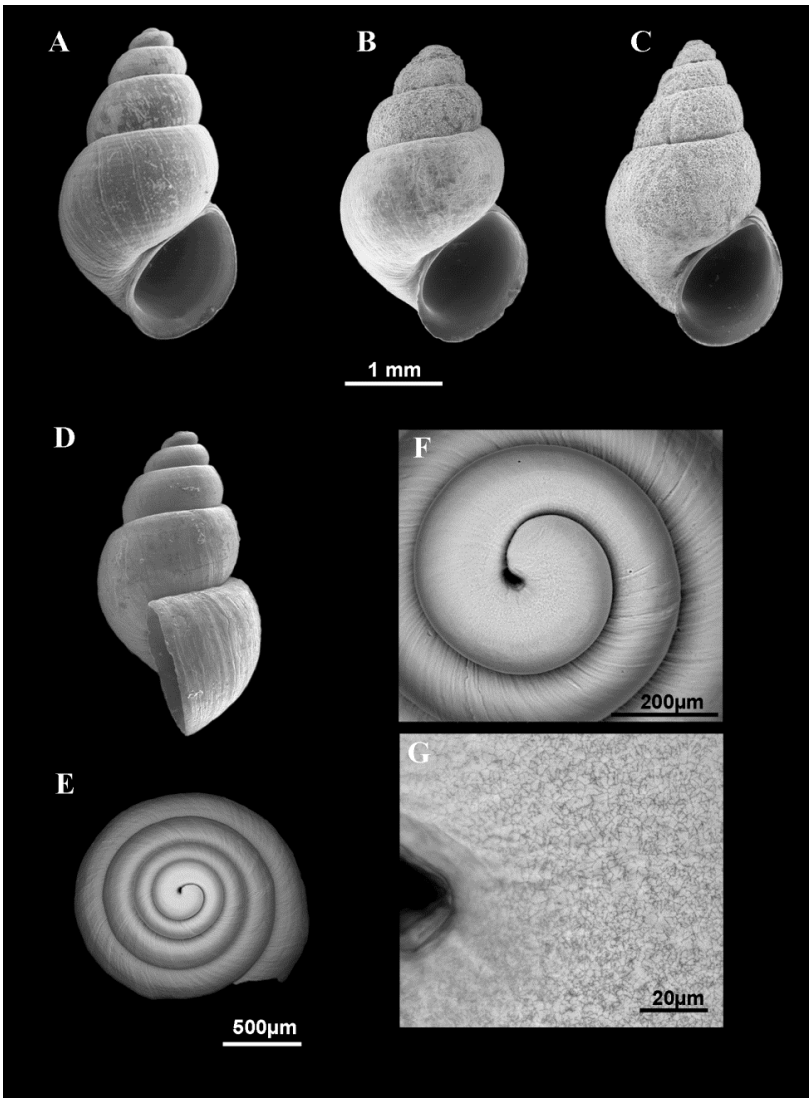


Figure 72. Shells of *Pseudamnicola (C.) marisolae*. **A**, **D-G.** Shells from Pilar del Mono Spring, Dúrcal, Granada (type locality). **B.** Shell from Fuente Grande, Alfácar, Granada. **C.** Palmones Spring, Padul, Granada. **A.** Holotype. **E-G.** Paratypes: protoconch and detail of the microsculpture of the protoconch.

Description

Shell yellowish periostracum with 4.5-5.5 spire whorls, height 4.60-3.25 mm (Figure 72A-C, Appendix III: Table 1); convex whorls with a very marked suture; body whorl occupies two-thirds of shell length; protoconch with around 1.6 spire whorls 500 μm wide and nucleus width approximately 150 μm (Figures 72E, F); protoconch microsculpture with grooves across entire surface (Figure 72G); peristome frontal, oval, complete, with a thin outer lip and thicker inner lip not in contact with body whorl; narrow umbilicus hidden behind inner lip of peristome; outer peristome simple and straight (Figure 72D).

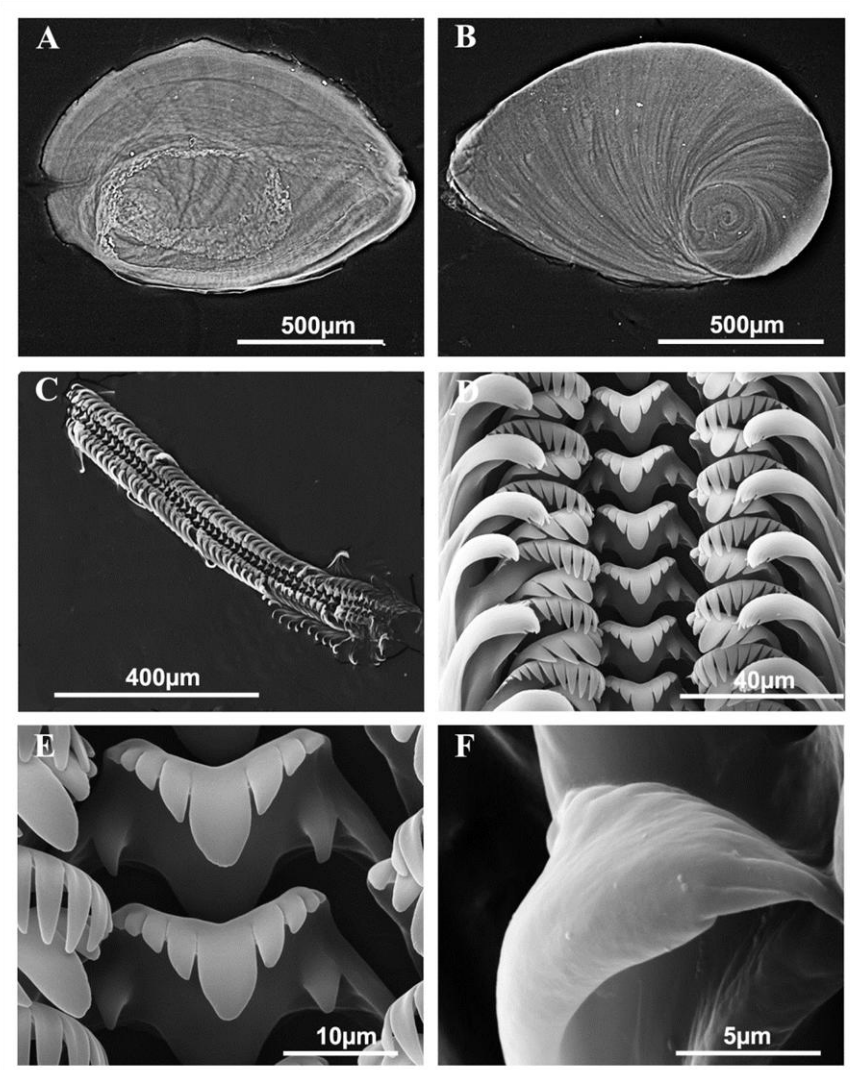


Figure 73. Operculum and radula of *Pseudamnicola (C.) marisolae* from Pilar del Mono Spring in Dúrcal, Granada (type locality). **A.** Internal side of the operculum. **B.** External side of the operculum. **C.** Radula. **D.** Rows of teeth of the radula. **E.** Central teeth. **F.** Detail of an outer marginal tooth.

Operculum translucent, with 3.5 spire whorls approximately (Figure 73B, Appendix III: Table 2); internal side has a convex edge and oval muscle attachment near nucleus (Figure 73A).

Radula with around 50 rows of teeth of medium size (22% of total shell length); eight times longer than wide (Figure 73C, Appendix III: Table 3); trapezoidal central tooth with a tongue-shaped median cusp and three or four pointed laterals (Figures 73D, E); lateral teeth longer than wide with two tapered lateral cusps and a rounded median cusp larger than laterals; inner marginal tooth with 11 cusps, approximately longer than those of outer marginal tooth (Figures 73D, F).

Pigmentation and anatomy. Head with dark brown pigment all over its surface except on the edge of snout and external edge tentacles (Figure 74D); dorsal region of foot also pigmented; pigment on neck clearer than on the head; snout as long as wide and tentacles shorter than snout; foot of intermediate size and anterior edge indented. Ctenidium with 20-24 well-developed gill filaments situated in the posterior section occupying most of the pallial cavity; osphradium located in opposite middle of ctenidium and two or three times longer than wide (Figure 74C, Appendix III: Table 4). Stomach with a posterior chamber larger than anterior chamber, relatively long caecum in a ventral position of the posterior chamber (Appendix III: Table 4); style sac shorter than stomach, longer than wide; oesophagus and intestine without pigment (Figure 74F); rectum lightly S-shaped in pallial cavity.

Female genitalia with a capsule gland with two regions (the anterior region more whitish) and a smaller albumen gland (Figure 74G, Appendix III: Table 5); bursa copulatrix pyriform J-shaped, relatively narrow after joining duct and later widely expanded; long and narrow bursal duct expanded near the point joining renal oviduct (Figure 74H); elongated seminal receptacle with short duct, slightly pigmented, attached close to base of renal oviduct; renal oviduct pigmented making one or two folds before the loop which is simple and long, later it continues straight from the loop until insertion of the bursal duct; pigmentation of renal oviduct fades strongly from loop to seminal receptacle (Figure 74H).

Male genitalia with a prostate gland four times longer than wide (Appendix III: Table 6); vas efferens entering the medial-posterior region and vas deferens exiting at the anterior (Figure 74E); long penis with a wide base, large pigment patch from middle section to tip and some folds in middle zone (Figure 74D); attached to central region of head, contains a wavy penial duct running on its right side.

Nervous system brown pigmented, darker on ganglia than on connectives and commissures; cerebral ganglia equal in size to pleural ganglia; supraoesophageal

connective four times longer than suboesophageal (Figure 74B, Appendix III: Table 7); RPG ratio 0.46 (moderately concentrated); straight oesophagus running beneath nervous system (Figure 74A).

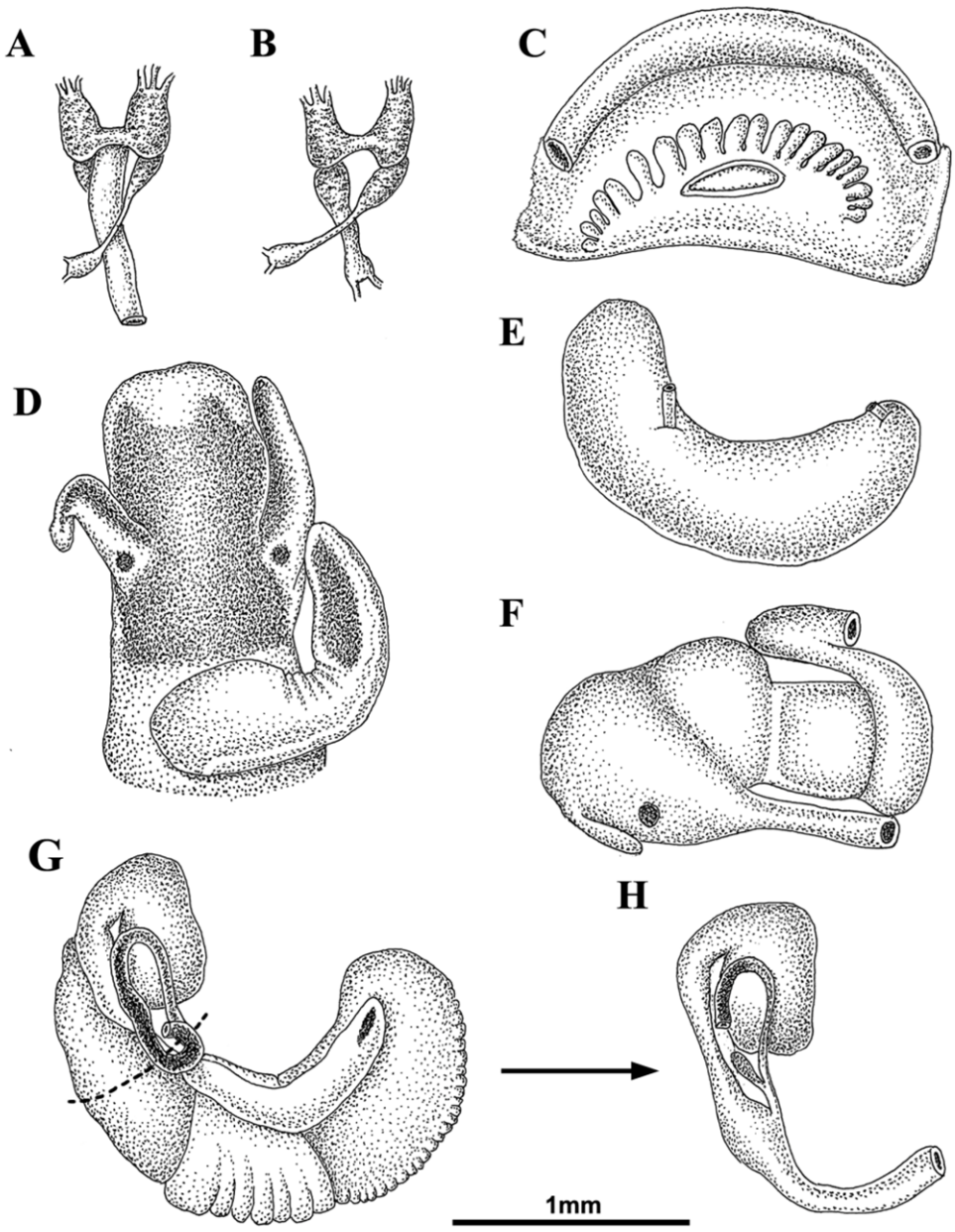


Figure 74. Anatomy of *Pseudamnicola (C.) marisolae* from type locality. **A**, **B.** Partial nervous system. **C.** Ctenidium and osphradium. **D.** Head of a male and penis. **E.** Prostate gland. **F.** Stomach. **G.** Female genitalia. Dotted line displays the pallial wall. **H.** Bursa copulatrix and seminal receptacle.

Remarks. Two characters differentiate *P. (C.) marisolae* from the other Iberian *Pseudamnicola (Corrosella)* species: the shape of the bursa copulatrix and the expansion of the bursal duct terminus close to the pallial oviduct.

Morphological differentiation between this species and *P. (C.) luisi*, whose distribution areas are very close, is complex because both species have large conic shells and a pigmented long penis. However, shells of *P. (C.) luisi* are larger than *P. (C.) marisolae* and its peristome finishes in a thicker inner lip than in *P. (C.) marisolae*. Furthermore, the penis of *P. (C.) luisi* is slender and around 0.30 mm longer than in *P. (C.) marisolae*; its base is also wider. The genetic distance between these two species is 6.2% for COI gene (Appendix IV: Table 1).

Despite *P. (C.) marisolae* and *P. (C.) falkneri* having conic shells, the two species differ according to a set of anatomical characters: the shell is more than 1.5 times taller in *P. (C.) marisolae*; radular formulae of teeth are very different in both species (Appendix III: Table 3); the bursa copulatrix is at least two times longer in *P. (C.) falkneri*; the penis has a pointed tip, small patch of pale pigment and attachment area occupies a central position on the neck in *P. (C.) falkneri*, but in *P. (C.) marisolae*, the penis has a rounded tip, a long patch of dark pigment and wider base located on the left side of the neck. The genetic distance in the COI gene between *P. (C.) marisolae* and *P. (C.) falkneri* is 8.7% (Appendix IV: Table 1).

With respect to other new species, their differentiation is clearer in terms of anatomy than shell shape. *Pseudamnicola (C.) marisolae* is the only new species whose lateral teeth formula is 2-C-2 (Appendix III: Table 3). The penis of *P. (C.) marisolae* has a wide base and a large dark patch of pigment in the distal region, whereas this pigmented area is smaller in *P. (C.) iruritai*, *P. (C.) manueli* and *P. (C.) bareai* and clearer in *P. (C.) andalusica* and *P. (C.) falkneri*. The whole seminal receptacle is pigmented in *P. (C.) marisolae*, whereas in *P. (C.) manueli*, *P. (C.) andalusica* and *P. (C.) iruritai*, pigmentation is restricted to the duct. The folded bursa copulatrix is J-shaped in *P. (C.) marisolae*, *P. (C.) manueli*, *P. (C.) andalusica* and *P. (C.) falkneri*, U-shaped in *P. (C.) bareai* and *P. (C.) luisi* and pyriform in *P. (C.) iruritai*.

Material for molecular analyses

Populations of *Pseudamnicola (Corrosella)* were examined in 50 localities in the Iberian Peninsula and in the Department of Var, Alpes-Maritimes, Southern France (Figure 75, Appendix II); these regions include the entire known range of the subgenus *P. (Corrosella)*.

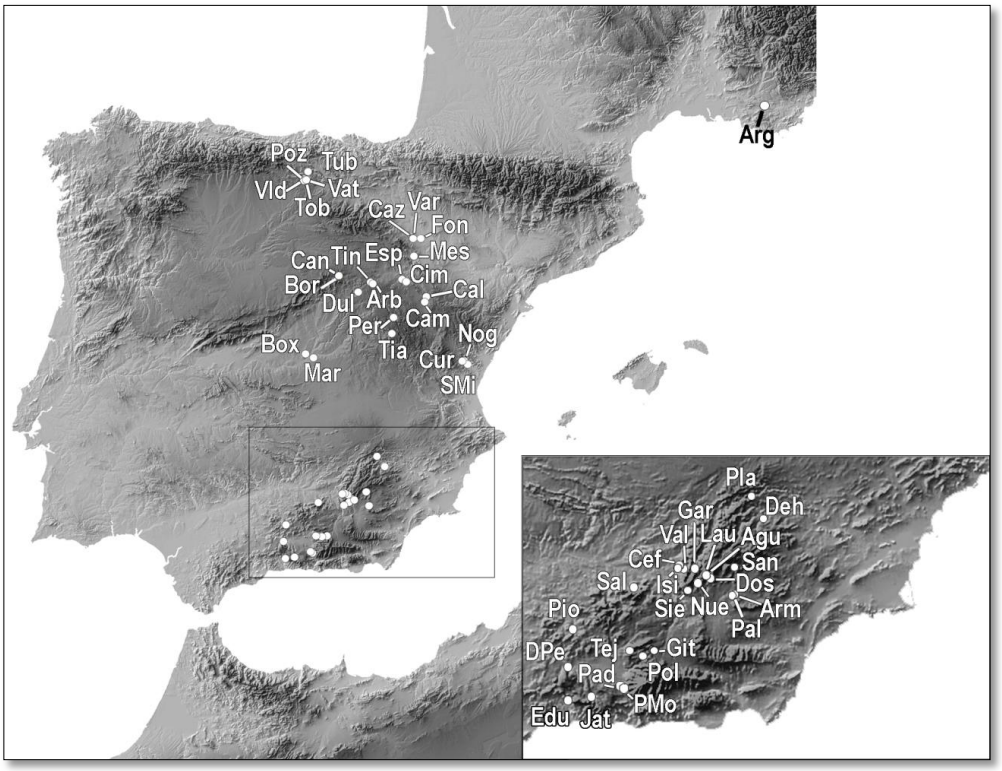


Figure 75. Distribution map of the *Pseudamnicola (Corrosella)* populations analyzed.

A final data set of 98 *P. (Corrosella)* spp. 16S, COI and 28S sequences from the 51 localities were analyzed as an ingroup (Table 11). The GenBank accession numbers assigned to them are: JF312218-28 (Delicado *et al.*, 2012), JQ067672-77 (Delicado and Ramos, 2012) and JX081805-76 for COI, JX081890-987 for 16S and JX081679-776 for 28S. Sequences of two specimens of the sister species *Pseudamnicola (Pseudamnicola) subproducta* were obtained and included in the analyses (GenBank accession numbers: JX081886-87 for COI, JX081988-89 for 16S and JX081777-78 for 28S). The species *Peringia ulvae* (Pennant, 1777) and *Mercuria emiliana* (Paladilhe, 1869) were used as outgroups (GenBank accession

numbers: *P. ulvae*, JX081889 for COI, JX081991 for 16S and JX081780 for 28S and *M. emiliana*, JX081888 for COI, JX081990 for 16S and JX081779 for 28S)

Table 11. Species, locality names and codes for species sequenced in this chapter.

Pseudamnicola (Corrosella) species	Locality	Code	*Pseudamnicola (Corrosella)* species	Locality	Code
astieri	Source d'Argens, Brue-Auriac, Alps Maritimes, France	Arg	*luisi*	La Teja spring, Sierra de Huétor, Spain	Tej
andalusica	La Salud spring, Albanchez de Mágina, Spain (type loc.)	Sal	*manueli*	Garganta stream, Nava de San Pedro, Spain (type locality)	Gar
	Eduardo spring, Alcaucín, Spain	Edu		El Valle stream, La Iruela, Spain	Val
	El Piojo spring, Almedinilla, Spain	Pio		El Céfano spring, La Iruela, Spain	Cef
ballestae n. sp.	Spring in Jatar, Spain (type loc.)	Jat		San Isidro ditch, Cazorla, Spain	Isi
bareai	Spring in Ermita de las Santas, Spain (type loc.)	San	*marisolae*	Pilar del Mono spring, Dúrcal, Spain (type loc.)	PMo
	Agüerillo, Castril, Spain	Agu		Spring in Padul, Spain	Pad
	El Laude, Castril, Spain	Lau	*navasiana*	Fonnueva spring, Bulbuente, Spain	Fon
	Fuente Nuevas, Castril, Spain	Nue		Ojos de Cimballa wetland, Zaragoza, Spain	Cim
	Siete Fuentes, Cuenca, Jaen, Spain	Sie		Spring in Mesones, Zaragoza, Spain	Mes
	La Plata spring, Riopar, Spain	Pla		Lago del Espejo, Nuévalos, Zaragoza, Spain	Esp
falkneri	La Armada spring, Orce, Spain	Arm		Tinte spring, Medinaceli, Soria, Spain	Tin
	Palo spring, Orce, Spain	Pal		Spring in Arbujuelo, Soria, Spain	Arb
	Spring in Castril, Spain	Cas		Spring in Peralejos de las Truchas, Guadalajara, Spain	Per

Pseudamnicola (Corrosella) species	Locality	Code	*Pseudamnicola (Corrosella)* species	Locality	Code
falkneri	Dos Caños spring, Castril, Spain	Dos	*navasiana*	Stream near Canalejas spring, Somolinos, Guadalajara, Spain	Can
	La Errá spring, La Dehesa, Spain	Deh		Source of Bornova river, Guadalajara, Spain	Bor
hauffei	Nogales spring, Benafer, Spain (type locality)	Nog		Dulce river, Cabrera, Guadalajara, Spain	Dul
	Curso spring, Navajas, Spain	Cur		Tía Perra spring, El Hosquillo, Cuenca, Spain	Tia
	San Miguel spring, Viver, Spain	SMi		Pozo Azul, Covanera, Burgos, Spain	Poz
hinzi	Vargas balsa, Borja, Spain	Var		La Toba spring, Tubilla del agua, Burgos, Spain	Tob
	Cazuelas spring, Borja, Spain	Caz		Valdeménez stream, Sedano, Burgos, Spain	Vld
	Spring of river park in Calamocha, Spain	Cal		Stream in Tubilleja, Burgos, Spain	Tub
	Prado spring, Caminreal, Spain	Cam		Stream in Valtubilla, Sedano, Burgos, Spain	Vat
iruritai	Don Pedro spring, Loja, Spain (type locality)	DPe		María spring, Ontígola, Toledo, Spain	Mar
luisi	La Gitana spring, La Peza, Spain	Git		Ditch in Borox, Toledo, Spain	Box
	Polvorista stream, Quéntar, Spain	Pol			

Phylogenetic inference

Mitochondrial data set. A combination of mitochondrial 16S and COI sequences resulted in an alignment of 102 specimens and 1170 positions (512 bp for 16S and 658 bp for COI). Of these, 790 positions were invariant, 317 were parsimony-informative and 63 were parsimony-uninformative. For both genes, all the phylogenetic inferences recovered the 11 formerly recognized species and revealed

the existence of a new undescribed species (here referred as *P. (C.)* sp.). Ingroup sequence divergences (measured as the mean of uncorrected *p* distances, Appendix IV: Table 1) varied: for COI, between 12% (*P. (C.) navasiana* vs. *P. (C.) ballestae* n. sp.) and 6.2% (*P. (C.) luisi* vs. *P. (C.) marisolae*) and for 16S, between 6.8% (*P. (C.) marisolae* vs. *P. (C.) bareai* and 1.0% (*P. (C.) hauffei* vs. *P. (C.) navasiana*). MP, ML and BI yielded similar topologies for both genes, clustering *P. (Corrosella)* species into three clades. The phylogenetic relationships of these clades were unresolved due to low bootstrap and posterior probabilities values observed at that level. For both genes, *P. (C.) hinzi* formed an independent lineage constituted by a single species, and its position was not clear along the phylogeny. The other two clades were composed of northern Iberian species (i.e., clade I) and southern Iberian species (i.e., clade III), respectively. Furthermore, the group *P. (C.) manueli* / *P. (C.) bareai* was supported within the southern clade mainly by 16S data. In the COI topology, this group was clustered, though poorly supported, within the northern clade.

Nuclear data set. Within *P. (Corrosella)* specimens, the 18S nuclear fragment sequences did not show genetic divergences among pairs of taxa. Only a difference of 0.2% (uncorrected distances) was found between *Corrosella* subgenus sequences and *P. (P.) subproducta*, the representative of the sister subgenus *Pseudamnicola*. Partial 28S gene sequences were included in a data matrix consisting of 102 specimens and 1037 bp containing 858 invariant sites, 115 parsimony-uninformative sites and 64 parsimony-informative sites. Average evolutionary divergences were lower than those for the mitochondrial genes. The ranges for *P. (Corrosella)* species were between 0% (*P. (C.) navasiana* vs *P. (C.) hauffei*) and 0.9% (*P. (C.) iruritai* vs *P. (C.) bareai*) (Appendix IV: Table 1). The BI analyses of 28S yielded a topology of four clades within the subgenus, with the following species: I) *P. (C.) falkneri*; II) *P. (C.) iruritai*, *P. (C.) andalusica*, *P. (C.) luisi*, *P. (C.) marisolae*, *P. (C.) ballestae* n. sp. and *P. (C.) hinzi*; III) *P. (C.) manueli* and *P. (C.) bareai* and IV) *P. (C.) astieri*, *P. (C.) navasiana* and *P. (C.) hauffei*. However, the phylogenetic relationship among clades remained unresolved.

Combined data set. After combining the three informative genes (COI, 16S and 28S), 2207 bp were examined; the reconstructed BI tree is depicted in Figure 76. This combined data set helped clarify the phylogenetic relationships between some groups of species, for instance, the position of *P. (C.) falkneri* and the group *P. (C.) manueli* / *P. (C.) bareai*. However, some relationships remained unresolved, in particular at two different levels of the phylogeny: 1) at the basal node of *Corrosella*, where a split into three clades is observed (Figure 76) and 2) within Clade III, where an unresolved relationship among *P. (C.) iruritai*, *P. (C.) andalusica*, *P. (C.) ballestae* n. sp., *P. (C.) marisolae* and *P. (C.) luisi* is observed.

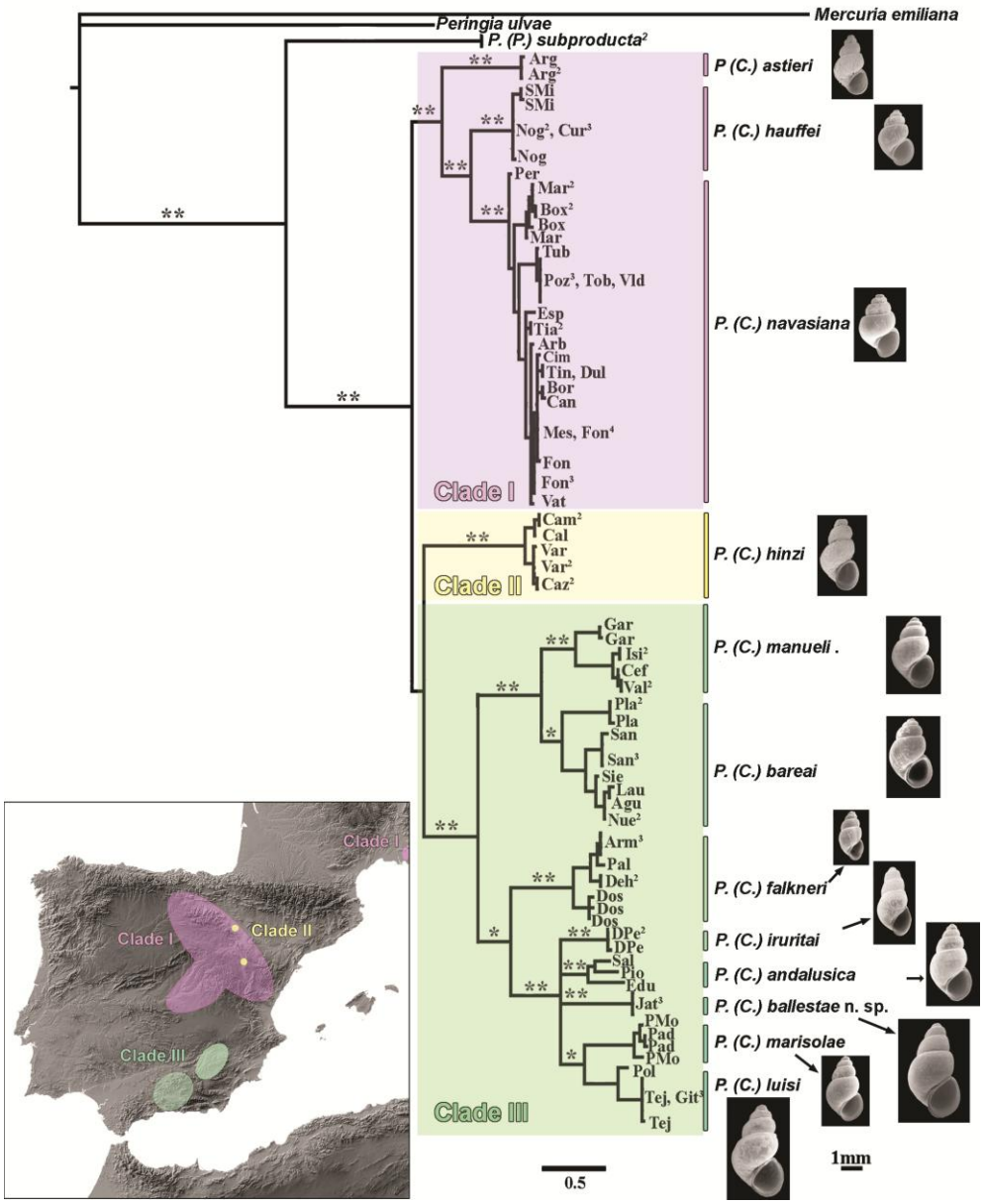


Figure 76. Bayesian phylogenetic tree of *P. (Corrosella)* spp. based on the combination of mitochondrial COI and 16S and nuclear 28S fragments. For locality code see Table 10. Supports of branches are given above species level as follows: ** represents bootstrap values ≥ 95% for ML and MP and Posterior Probabilities ≥ 0.95; * indicates bootstrap values ≥ 95% for ML, 80 ≤ * < 95% for MP and Posterior Probabilities ≥ 0.95.

None of the three individual analyses were able to resolve these inconsistent nodes, yet they supported the species entities and the existence of three lineages grouping those taxa. Analysis of the mitochondrial and the nuclear 28S markers combined showed that the *P. (C.) manueli* / *P. (C.) bareai* group was within clade III

(consistent with the mitochondrial reconstruction but not with the 28S gene reconstruction).

Divergence time estimates

The *BEAST tree (Figure 77) showed the estimated divergence times for *P. (Corrosella)* species. Although a strict molecular clock approach involves less

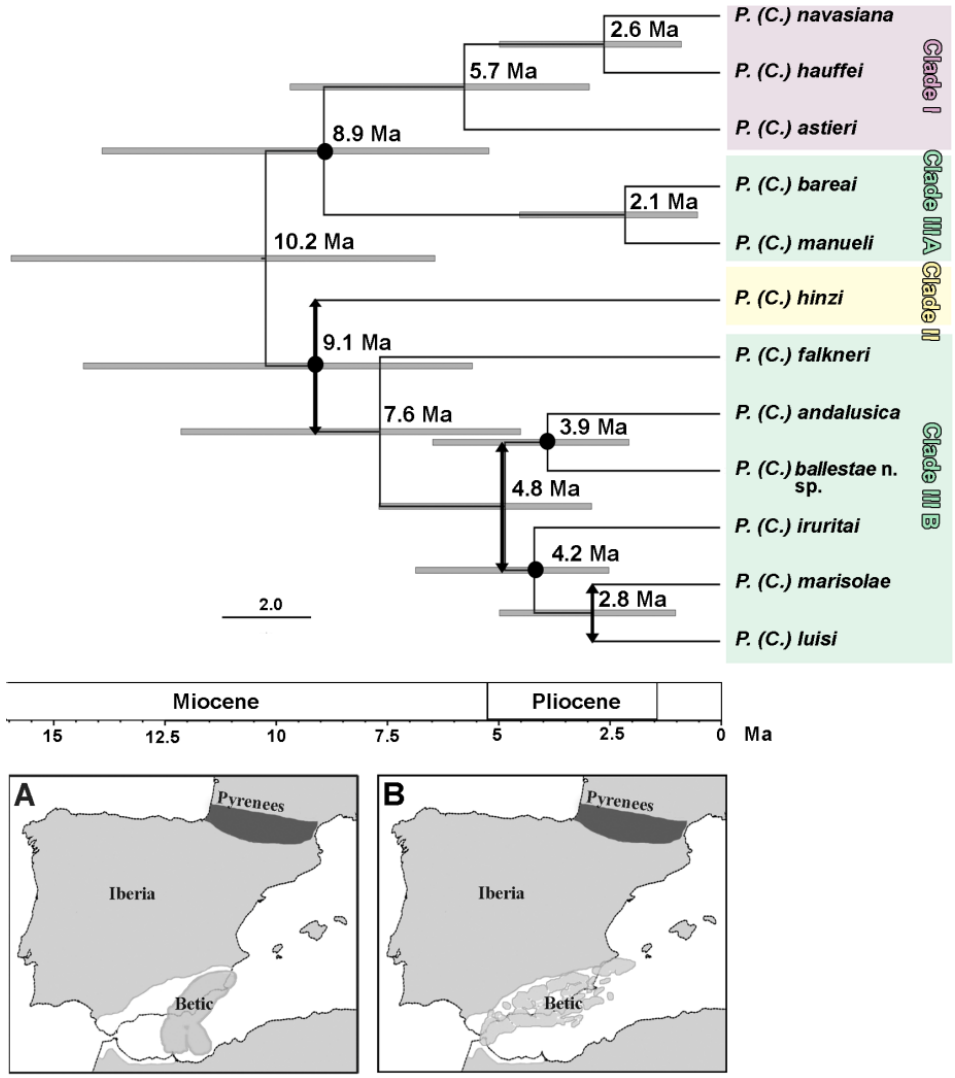


Figure 77. Chronogram of the *P. (Corrosella)* spp. obtained with *BEAST using published biogeographical calibration points for hydrobiids. Filled circles on nodes indicate posterior probabilities less than 0.50. Bars represent 95% HPD (highest posterior density) intervals. Black arrows on branches indicate changes in the constancy of the diversification rates resulting of the relative rate test. **A.** Reconstruction of the Iberian Peninsula 15 Ma, following Rosenbaum *et al.* (2002). **B**. Reconstruction of the Iberian Peninsula 6.7 Ma, simplified from Benson *et al.* (1992) and Barbadillo *et al.* (1997).

parameters and thus, usually a higher likelihood, the likelihood values and some of the ESS values here were higher under relaxed clock conditions (-ln L = 6860.90 and 6865.59 for relaxed and strict clock-like models, respectively). The substitution rates for each gene partition, inferred from the COI rate, were 0.3 ± 0.001% for 16S and 0.03 ± 0.0001% for 28S (with an ESS higher than 5,000).

The topology of the *BEAST tree generated using the relaxed molecular clock model was the same as the MP, ML and BI phylogenetic reconstruction, except for the position of the *P. (C.) bareai* / *P. (C.) manueli* group. In this case, the *P. (C.) bareai* / *P. (C.) manueli* group is included within clade III, whereas in the ultrametric tree, its position is ambiguous since the basal nodes registered low supports at that level. Therefore, referring to this last topology the subgenus was here divided into four clades: clades I and II, coinciding with those of the phylogenetic inferences, while clade III split in clades IIIA and IIIB, both gathering southern species. All of the methods supported the monophyly of *Corrosella*, and two possible radiations within it, occurred approximately 10 Ma and 5 Ma. Relative rate tests identified more rapid diversification at these two levels, specifically along the branches splitting clade II and clade III and the species *P. (C.) ballestae* n. sp. and the group *P. (C.) luisi* / *P. (C.) marisolae* (see Figure 77). Therefore, this test rejected a model of constant rate diversification. Finally, the last diversification process took place in both northern and southern clades approximately 2 Ma, i.e., in the early Pleistocene, according to the ultrametric tree.

Table 12. Mantel test parameters resulting of comparing genetic distance matrix (uncorrected p-distances) of each gene partition with distance matrices of abiotic variables such as water conductivity, altitude of the locality and geographic distances between two populations. The value of n represents number of localities included in each analyzed correlation. *r* is described as correlation coefficient and *p* the statistical significance.

Genetic distances	Independent variable	n	r	p
COI	Conductivity	36	0.05	0.302
	Altitude	50	0.15	0.020
	Geographic distance	50	0.51	0.001
16S	Conductivity	36	0.02	0.488
	Altitude	50	0.05	0.108
	Geographic distance	50	0.64	0.001
28S	Conductivity	36	0.26	0.074
	Altitude	50	0.38	0.002
	Geographic distance	50	0.18	0.002

Contrary to the other phylogenetic inferences, only the reconstruction by coalescence of the 16S fragment had posterior probability values at the basal radiation higher than 0.95, thus placing *P. (C.) hinzi* within clade I (with species from the northern Iberian Peninsula), and the *P. (C.) bareai* / *P. (C.) manueli* group within clade III (with species from the southern Iberia Peninsula).

Diversification factors

In the absence of outgroups, Mantel tests revealed no statistical correlation between genetic divergences and conductivity of the water or altitude (Table 12, Figure 78) for the three genes. A single significant correlation was found between geographic and genetic distances (P values shown in Table 12), showing an isolation by distance pattern.

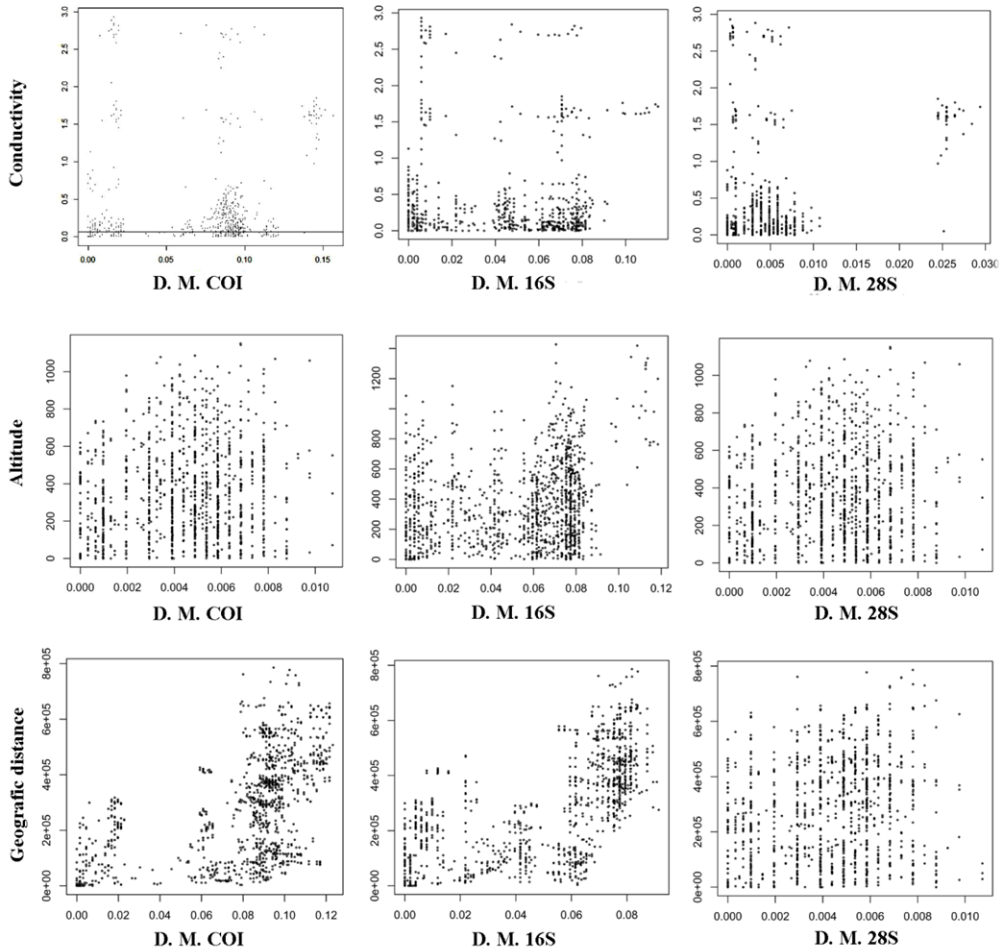
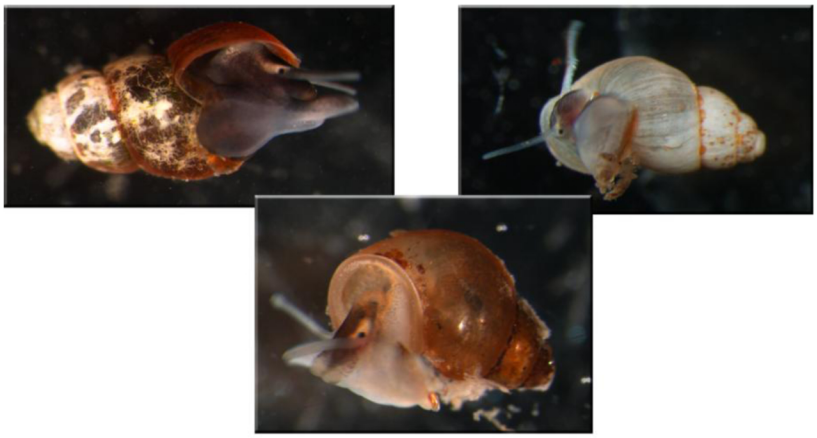


Figure 78. Mantel test scatterplots showing the correlation between the genetic distance matrix of each studied gene and distance matrices of conductivity, altitude and geographic distances.

After plotting the 128 collecting points on an Iberian Peninsula map and using GIS to extract additional data, such as the age, petrology and pH of soils, we observed that these parameters were often variable, not only between species of a clade but also within the same species. We also checked the distribution of those points along river basins, but even if some trends were apparent, some species (for instance, *P. (C.) navasiana*) inhabit more than one basin and in some cases, more than one species occupy a single river (although never coexisting at the same locality). For instance, in the Guadalquivir River, all clade III species have been found except for *P. (C.) marisolae*, which is found in a small independent river basin.

3. PATRONES FILOGENÉTICOS E HISTORIA EVOLUTIVA DEL GÉNERO ***PSEUDAMNICOLA***



To assess *Pseudamnicola* evolutionary relationships, we examined a total of 202 specimens from 95 localities belonging to the genus and two outgroup species, *Peringia ulvae* and *Mercuria emiliana*. Ingroup localities included 20 for *P. (Pseudamnicola)* spp. from the Ibero-Balearic region (Table 9), 51 for *P. (Corrosella)* spp. (Table 11) and 24 for additional *P. (Pseudamnicola)* populations from other areas of the Mediterranean basin (Table 13). Some of the species from the 24 Mediterranean localities were previously collected and identified by local hydrobiid experts.

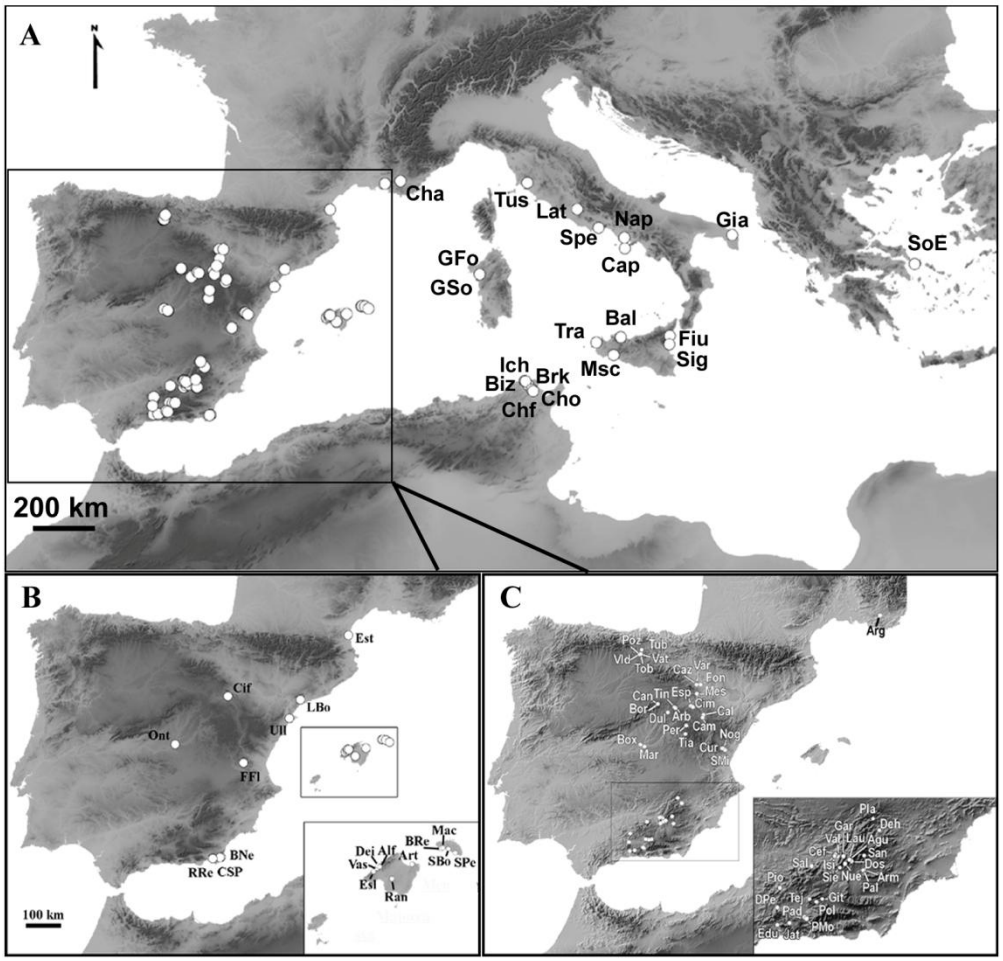


Figure 79. Distribution maps of samples sequenced in this work. Map **A** shows the location of the additional Mediterranean basin localities studied in this chapter. Maps **B** and **C** show the localities previously introduced in the Results. Code sites explanations are in Tables 9, 11 and 13.

Table 13. Additional *Pseudamnicola* species and localities from the Mediterranean basin not previously analyzed in the Results, with their corresponding locality codes.

Pseudamnicola (Pseudamnicola) species	Locality	Code
subproducta	Chalissane St. Chamas, Etang de Berre, Bouches du Rhône, France	Cha
moussoni	Spring in Via Marrucella, Capri Island, Italy	Cap
	Spring between Valle Bucceri and Valle Ficarella, Isle of Marettimo, Favignana, Trapani, Sicily, Italy	Tra
	Spring on Menfi-Sciacca Road, Sicily, Italy	MSc
	Spring near the trough of Balateddi, Portella Road, Sicily	Bal
	Spring near the Signalmans, Sicily, Italy	Sig
conovula	Stream near Sperlonga, Italy	Spe
	Grotta Fontana, Cossoine, Sardinia, Italy	GFo
	Spring in Via Marrucella, Isle of Capri, Italy	Cap
	Spring on Menfi-Sciacca Road, Sicily, Italy	MSc
	Spring on the left bank of T. Biedano, 100m upstream of the bridge under Blera, Latium, Italy	Lat
orsinii	Fiumefreddo, Sicily, Italy	Fiu
macrostoma	Marmari, South Evia, Greece	SoE
sp 1	Spring under the road to Baratti, near the Necropoli Etrusca, Com. Piombino, Grosseto, Tuscany, Italy	Tus
sp 2	Spring near Napoli, Italy	Nap
	Giammatteo Creek, Lecce, Italy	Gia
	Borkane Ditch, Tunisia	Brk
	Ditch towards the north of Ichkeul, Tunisia	Ich
	Stream near Bizerte, Tunisia	Biz
sp 3	Grotta Fontana, Cossoine, Sardinia, Italy	GFo
	Grotta Sorigalza, Cossoine, Sardinia, Italy	GSo
sp 4	Borkane ditch, Tunisia	Brk
	Chafrou River, Tunisia	Chf
	Stream near Chaouat, Tunisia	Cho

Mitochondrial data set. After combining COI and 16S fragments, a total of 1170 characters was obtained, of which 658 were for COI and 512 for 16S. The COI data set contained 257 variable sites, 236 of which were parsimony informative. The 16S data set contained 169 variable sites, 118 of which were parsimony informative. In all reconstructions with this data set, the genus *Pseudamnicola* is recovered as a monophyletic group that seems to be composed of three well-supported clades (Figure 80). However, the relationships between these groups are not obvious from the study of these mitochondrial genes, and this is reflected in the position of *P. (P.) gasulli* in the different tree topologies. In the COI topology, *P. (P.) gasulli* is clustered within the subgenus *P. (Corrosella)* with high support values in all analyses; in contrast, in the 16S reconstruction, this species is more closely related to the other *P. (Pseudamnicola)* species, though this is poorly supported in the ML analysis. Moreover, the reconstructions of both genes displayed different topologies. Overall, the relationships among *Pseudamnicola* spp. seem better resolved in the COI analysis (Figure 80). The percentage of sequence divergence was higher for COI than for 16S, with maximum genetic divergence of 16.6% for COI (between *P. (P.) granjaensis* and *P. (C.) marisolae*) and 10.6% for 16S (between *P. (P.) conovula* and *P. (C.) ballestae* n. sp.).

Nuclear data set. The 28S alignment consisted of 204 sequences each with 1057 characters. Of these, 815 were invariant, 54 parsimony uninformative and 188 parsimony informative. Interspecific genetic variation ranged from 0% (as between *P. (P.) beckmanni* and *P. (P.) granjaensis*) to 6.9% (between *P. (P.) gasulli* and *P. (P.) artanensis*). The ML and BI topologies recovered *Pseudamnicola* as a monophyletic group, clustering *P. (P.) gasulli* with the other *P. (Pseudamnicola)* species, with a bootstrap value near 80% for ML. All the species are grouped as a polytomy within their corresponding clades (Figure 80).

Combined data set. There are obvious disparities between the nuclear and mitochondrial gene trees related to the phylogenetic position of *P. (P.) gasulli*. Because of these disparities, the ILD test showed no congruence between the mitochondrial and nuclear data. Nevertheless, as indicated previously (see Results, Chapter 1, page 99), this does not mean than the resulting phylogeny is incongruent. As shown in Figure 80, the phylogeny of the genus is well resolved at basal nodes; however the evolutionary relationships among the three lineages constituting *Pseudamnicola* are ambiguous. The biogeographic pattern reflected by the MP, ML and BI topologies is more explicit in the subgenus *P. (Corrosella)* than in

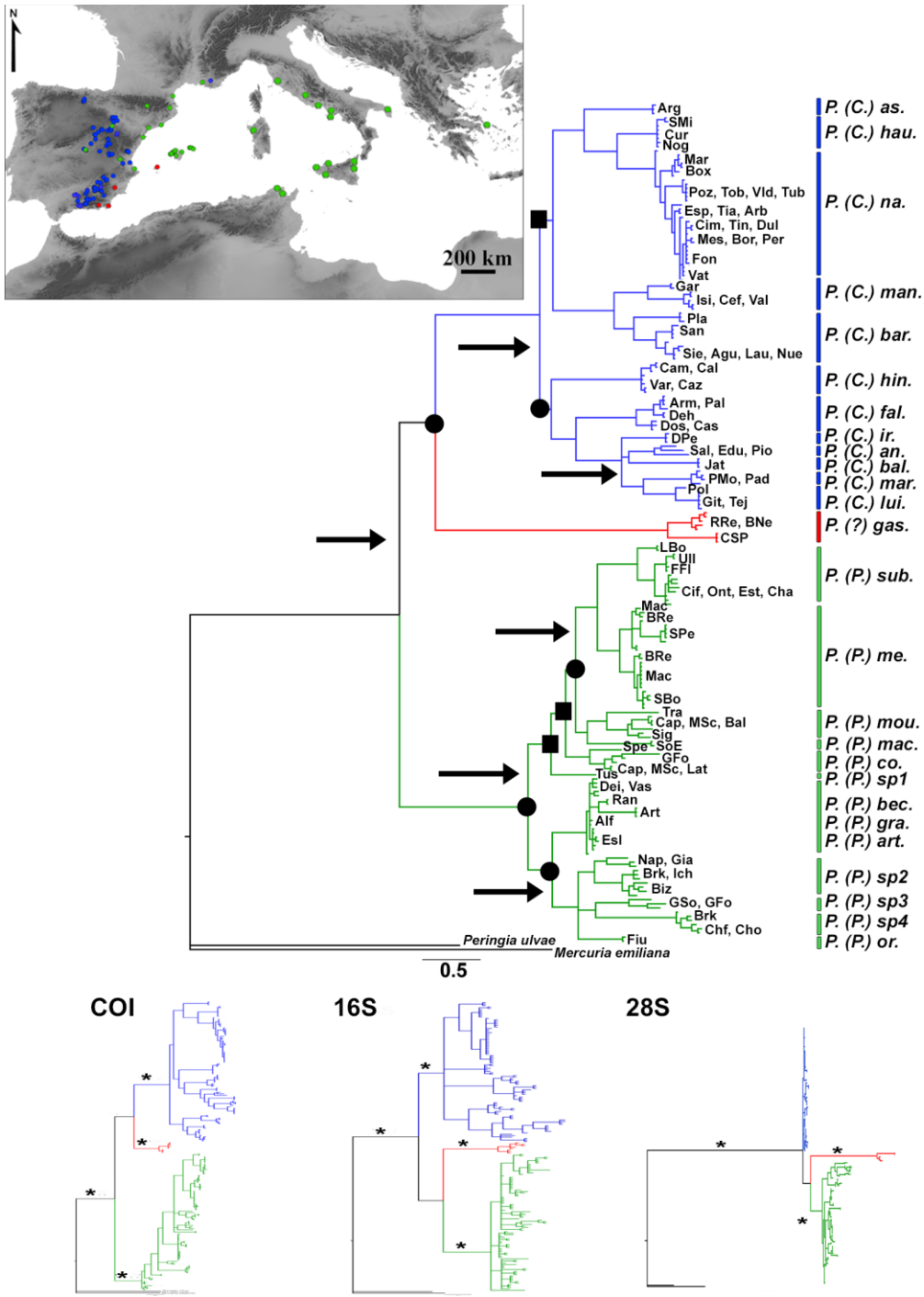


Figure 80. Phylogenetic relationships of *Pseudamnicola* spp. based on Bayesian inference of the combined data set (top) and the mitochondrial fragments COI and 16S (bottom). The topology of the nuclear 28S gene based on the Maximum Likelihood analysis is also shown (bottom). Black circles represent MP and ML bootstraps ≤ 90% and posterior probabilities ≤ 0.9, black squares represent MP and ML supports ≤ 90%, but posterior probabilities > 0.9 and asterisks indicate MP and ML supports > 90% and posterior probabilities > 0.9. Arrows point to branches with no rate constancy (result from Relative Rate Test).

P. (Pseudamnicola). In contrast to *P. (Corrosella)* (see Chapter II for biogeographic pattern), *P. (Pseudamnicola)* spp. do not show an apparent biogeographical distribution model, which is reflected by several observations, for instance: i) the species *P. (P.) beckmanni* (from Majorca) is genetically closer to the species occurring in Tunisia, Sicily and mainland Italy than to *P. (P.) meloussensis* (from Minorca), which is found in the same archipelago of islands; ii) the same species of *P. (Pseudamnicola)* are found in different regions, such as *P. (P.) conovula*, in mainland Italy, Sardinia and Sicily; iii) some species seem to live sympatrically in the same locality, such as *P. (P.) conovula* and *P. (P.) moussoni*, which both inhabit the same spring on the island of Capri, Italy.

STATISTICAL DISCRIMINATION OF THE THREE PHYLOGENETIC CLUSTERS OBTAINED FOR *PSEUDAMNICOLA*

The grouping of the three lineages within *Pseudamnicola* (Figure 80) resulted in highly significant (p-value 0.0001) discrimination models for both shell morphometry and anatomical variation. Accordingly, on the scatterplots, three clusters were observed (Figures 81, 82), although the distinction among groups was more obvious in the analysis based on anatomical measurements than on shell dimensions.

Conchological differences among *Pseudamnicola* lineages were studied by the canonical discriminant function analysis employing eight out of ten shell variables (neither WAW nor NSW (see Table 6 for abbreviations) followed a normal distribution, even after applying a logarithmic transformation). In a total of 492 measured shells, 70.3% were correctly classified according to the preliminary groups established for the analysis. Two highly significant discriminant functions were obtained (Wilk's lambda = 0.4852, F (14, 966) = 30.058, $p < 0.0001$). The variables that contributed the most in the analysis were SL/SW, WPW, AH, AmW and AmL. The most discriminant variables (highest weight) for the first function, which accounted for 96.2% of the explained variance, were SL/SW and WPW. For the second function, the most discriminant variables were AH, AmW and AmL.

For anatomical features, all the discriminant models were significant, however the distinction between groups is more obvious in the analysis of female features (Figure 82A). In the female model, two highly discriminant functions were found (Wilk's lambda = 0.0631, F (50, 118) =7.037, $p < 0.0001$). Of the females measured, 97.7% were correctly classified according to the groups originally designated. The most discriminant variable for the first function, which accounted

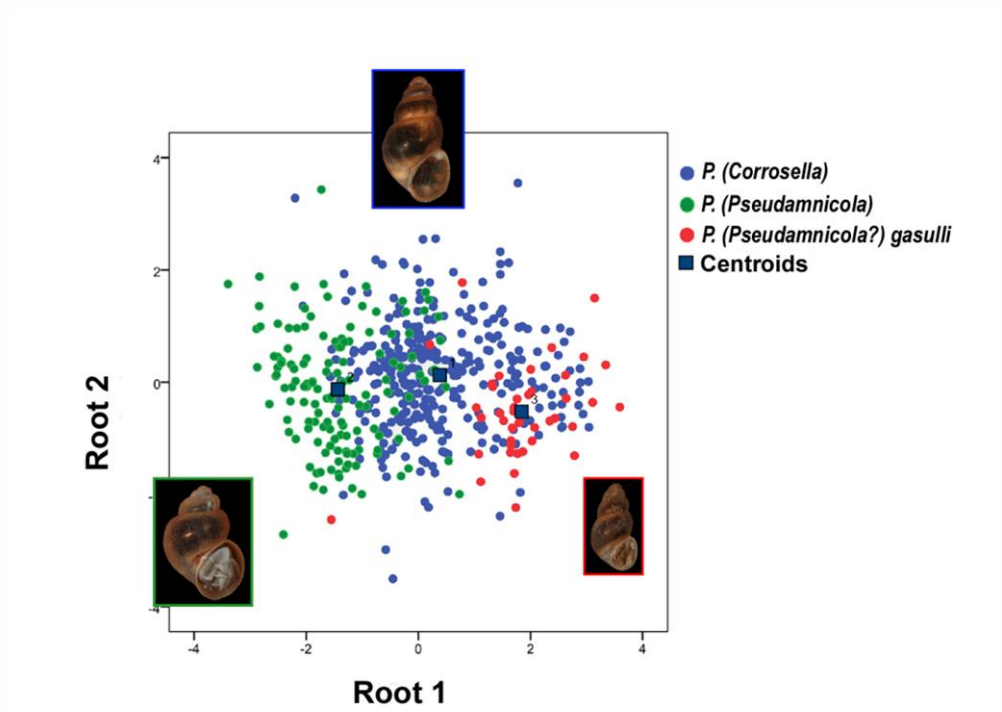


Figure 81. Plot of the two first roots of the canonical discriminant analysis based on conchological dimensions of all *Pseudamnicola* spp. included in this work.

for 65.6% of the explained variance, was SRL, followed by LSupG (with much less correlation than SRL with Root 1). In the second function, the variables with the most correlation were: BcL, CgL and PoL. Three well-defined groups were obtained each corresponding to one of the three lineages recovered by phylogenetic analyses (Figure 80).

Two highly significant discriminant functions were found in the analysis for male features (Wilk's lambda = 0.1306, F (44, 172) = 6.908, $p < 0.0001$). All the variables held a normal distribution except for OsW and LPRG, both of which reached the normality with a logarithm transformation. For the first function, which accounted for 84.5% of the explained variance, the characters with the most correlation were PrL and LSubC, respectively. For the second function, PW followed by SsW with much less correlation were most correlated. Of the males measured, 94.6% were correctly grouped within the three *Pseudamnicola* clusters (Figure 82B).

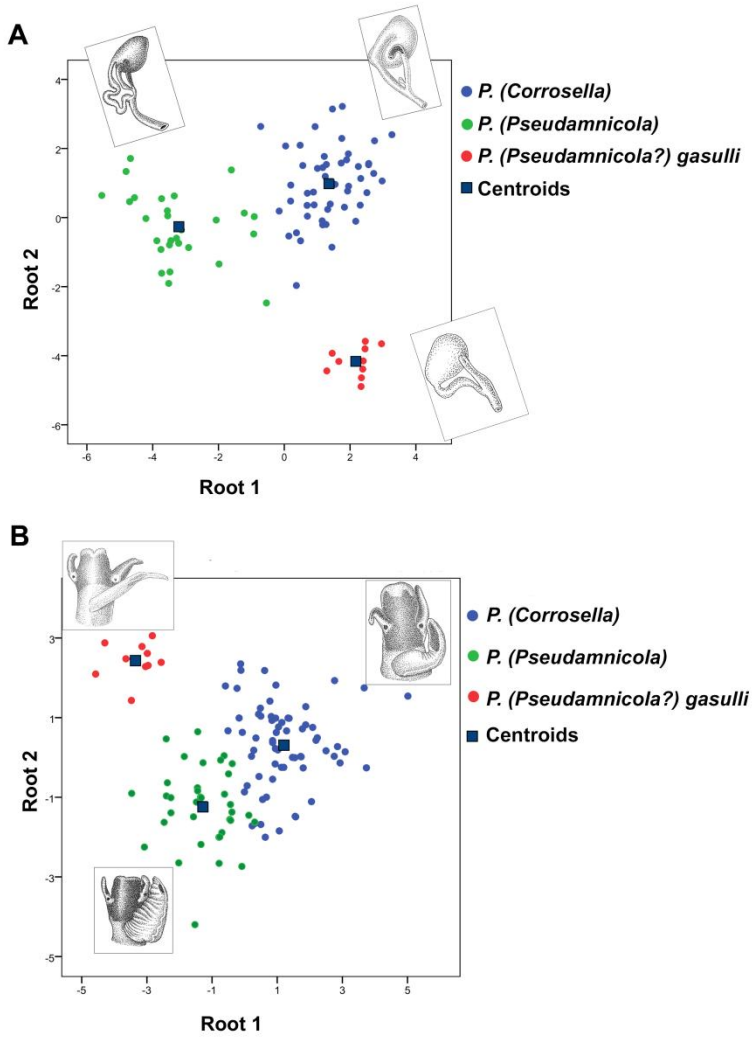


Figure 82. Plot of discriminant scores obtained by DFA of anatomical variables for females (**A**) and males (**B**) of *Pseudamnicola* spp. Schematics represent the bursa copulatrix and seminal receptacle of female genitalia on plot A and the head and penis of males on plot B.

DIVERGENCE TIME ESTIMATION

The non-constancy of the substitution rate has been demonstrated since the relative rate test showed significance differences in evolutionary rates between tree branches along the phylogeny (branches that showed significant differences in evolutionary rates are highlighted in Figure 83). Thus, the model of constant-rate of diversification may be rejected and the relaxed molecular clock approach applied.

After performing the analysis, the substitution rates inferred for each partition (substitution/MY) were 0.3% for 16S and 0.17% for 28S. Given that the ucld.stdev values and coefficients of variation in all the markers were greater than zero, evolution has not been clock-like for these genes (or markers). Furthermore, 28S had higher deviation from a strict clock model as its coefficient of variation was greater than 1.

The topology of the chronogram with corresponding confidence intervals analyzed in *BEAST is shown in Figure 83. Using a calibration of 0.81 ± 0.24% substitution/MY for the COI fragment, and rate estimations for 16S and 28S, the most basal split leading to the three major clades within *Pseudamnicola* is calculated to have occurred ca. 22 Ma (28-17 Ma), during the Upper Miocene. Contrary to the rest of the phylogenetic inferences, the reconstruction by coalescence showed high posterior probabilities at the level of these three lineages, associating the species *P. (P.) gasulli* to the *P. (Pseudamnicola)* lineage and dating their subsequent split ca. 17 Ma (23-13 Ma). However, this result requires further investigation since currently only one species composes the clade of *P. (P.) gasulli*. Thus, this fact may be why the relative rate test shows significant differences on evolutionary rates when comparing the *P. (P.) gasulli* lineage with each of the other two lineages, but shows no significant differences when comparing *P. (Corrosella)* and *P. (Pseudamnicola)*. Nonetheless, the topology of each of the lineages displays dissimilarities in tempo and mode of species diversification.

The age of the most recent common ancestor of *P. (Corrosella*) spp. is estimated to be older (ca. 13 Ma) than the age of the *P. (Pseudamnicola)* spp. ancestor, excluding *P. (P.) gasulli* (ca. 6 Ma). Consequently, all of the cladogenetic events that occurred during *P. (Corrosella)* evolution are likely older than those for *P. (Pseudamnicola)*. Moreover, these two lineages may have experienced a radiation event near the base of each lineage, since the relative rate test shows rapid diversification at basal levels of both clades. In any case, the radiation event that occurred during the origin of *P. (Pseudamnicola)* involves more species and a relatively shorter period of time (ca. 8-3 Ma), characterizing the major cladogenetic event for this group. Rate constancy is rejected by the relative rate test in most of the comparisons between branches within the *P. (Pseudamnicola)* lineage, which may affect the split frequencies (probably accelerated) and thus justify the low BBP values at the affected nodes.

The inclusion of additional *P. (Pseudamnicola)* sequences to the *BEAST analyses did not substantially change the divergence times previously estimated for the three major cladogenetic events leading to diversification within the *P. (Corrosella)* clade (see Chapter 2 of the Results). In the analysis here, these events are estimated to have occurred slightly earlier (ca 12 Ma, 6 Ma and 3 Ma) to the

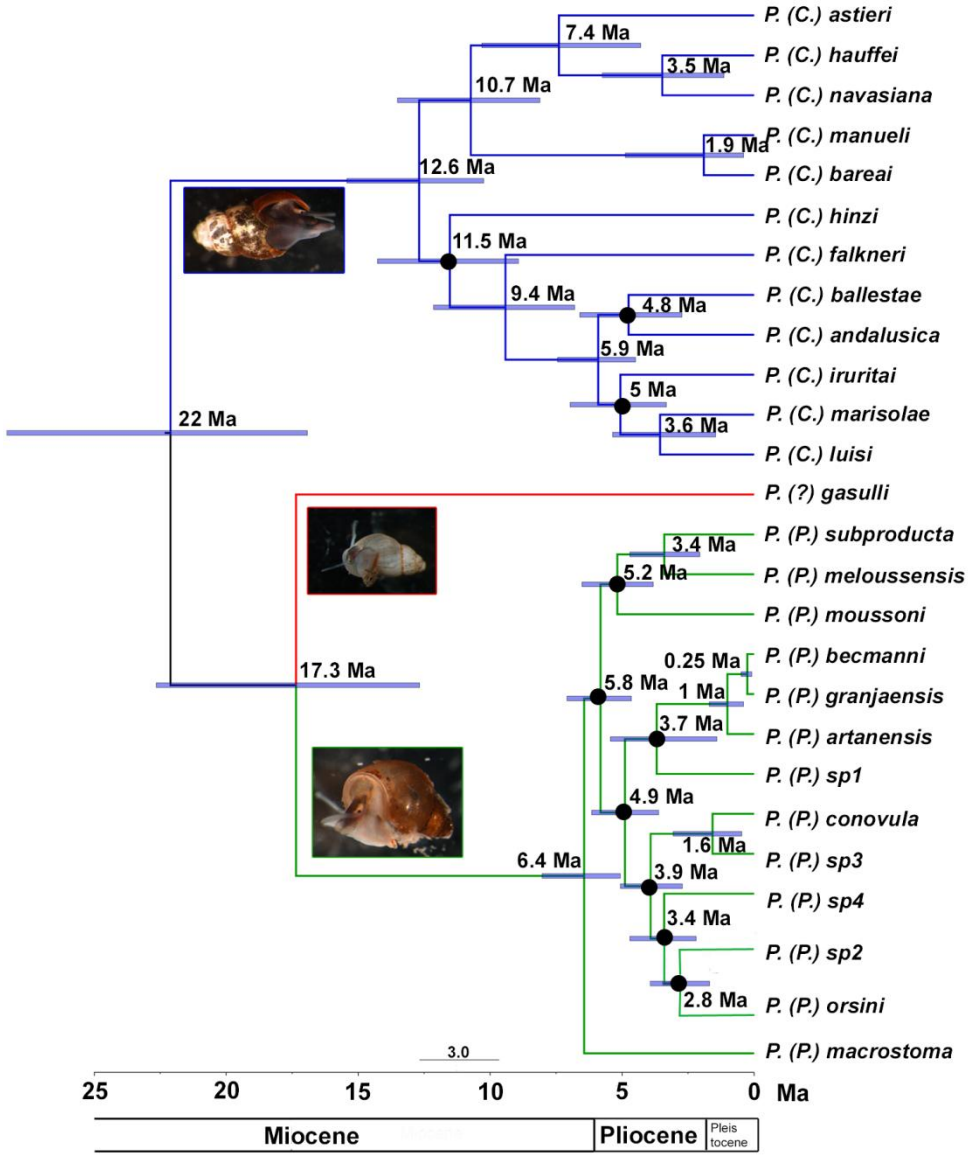


Figure 83. Ultrametric tree obtained with *BEAST based on the combined analysis of COI (using a rate previously calibrated for hydrobiids) and the ribosomal fragments 16S and 28S (using rates estimated in this analysis). At nodes where *BEAST posterior probabilities (BPP) are not given, the node is supported by BPP ≥ 0.90; black dots indicate BPP < 0.90. Bars at nodes represent confidence intervals. Units of scale bar: Ma.

ca. 10 Ma, 5 Ma and 2 Ma estimated in Results, Chapter 2. The most recent splits allopatric sibling species for *P. (Pseudamnicola)* spp. are likely to have occurred from ca. 5 Ma to 0.08 Ma. All of these splits appear not well supported, possibly due to changes in the substitution rate towards the present.

Mantel tests performed separately for each subgenus (Tables 10, 12 and 14) reveal no correlation between the genetic distance matrix and physical variables, such as conductivity and altitude, but a pattern of isolation by distance is found. However, when both subgenera are included in the analysis, Mantel tests show significance with the three examined variables, namely conductivity, altitude and geographic distance (Table 14, Figure 84). Despite this, it seems that conductivity and altitude only have minor influences, compared to geographic distance, on the divergence of the subgenera for the COI and 16S genes, while conductivity has more influence for the 28S gene, followed by geographic distance.

Table 14. Mantel test parameters of correlations between the genetic distance matrix of each gene fragment and distance matrices of the abiotic variables of water conductivity, altitude and geographic distances between two localities. The value n represents the number of localities included in each correlation, *r* is the correlation coefficient and *p* is the statistical significance.

Genetic distances	Independent variable	n	r	p
COI	Conductivity	52	0.06	0.001
	Altitude	88	0.32	0.001
	Geographic distance	88	0.44	0.001
16S	Conductivity	52	0.25	0.001
	Altitude	88	0.38	0.001
	Geographic distance	88	0.44	0.001
28S	Conductivity	52	0.45	0.001
	Altitude	88	0.20	0.001
	Geographic distance	88	0.39	0.001

As in the previous two results sections, after displaying on a map the 181 collecting points for *Pseudamnicola*, no pattern of distribution is found according to the age, petrology and pH of soils. That is, the features of the soil do not seem to affect the occurrence of each subgenus or each species. Nonetheless, we discovered or confirmed that, general, *P. (Corrosella)* spp. inhabit mountainous regions, in nutrient-poor springs and streams usually with low levels of conductivity (Appendix II), whereas *P. (Pseudamnicola)* spp. occur in locations of low altitude (generally near the coast) and higher levels of conductivity. Although it seems that the species

P. (P.) gasulli is genetically distinct from both subgenera, its ecological conditions and habitat features are more similar to those of *P. (Pseudamnicola)* spp.

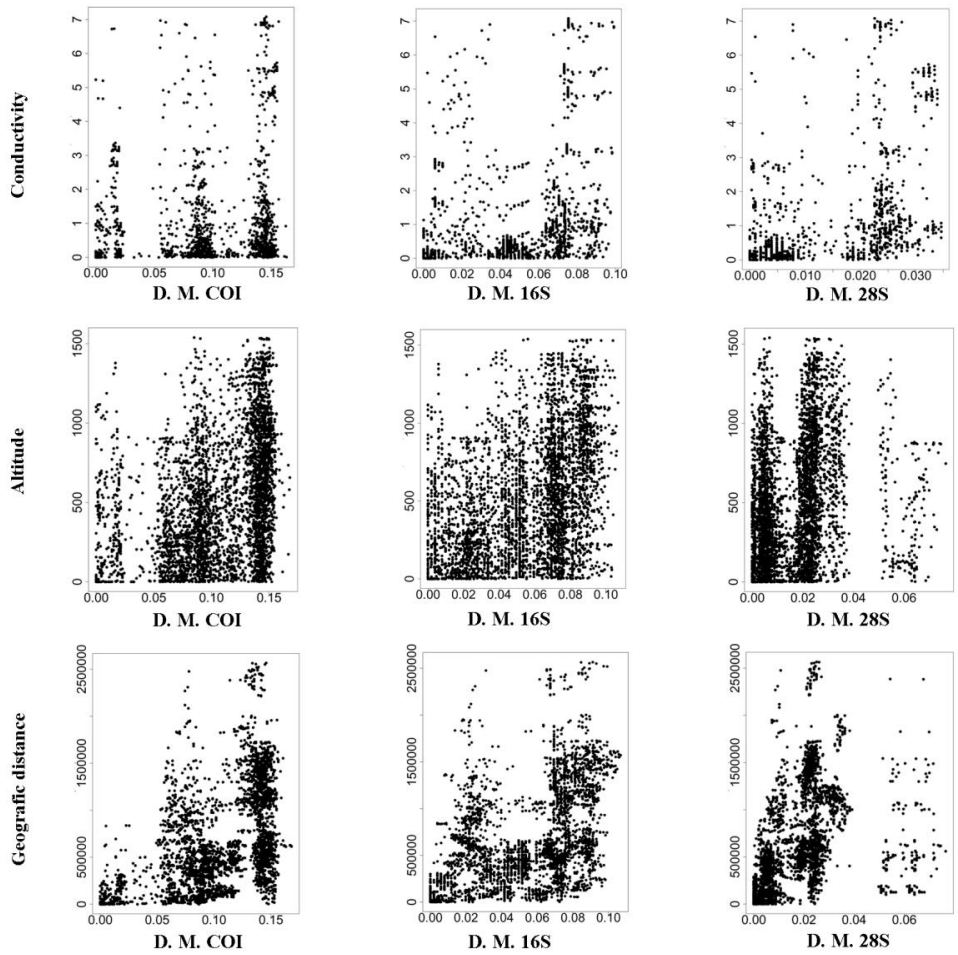


Figure 84. Mantel test scatterplots showing the correlation between the genetic distance matrix (D. M.) of each studied gene and distance matrices of conductivity, altitude and geographic distances.

DISCUSIÓN

The systematic analysis of hydrobiid gastropods is challenging because their small size and simple anatomical structures make it difficult to establish morphological species boundaries. Iberian hydrobiids also have cryptic species, which further complicates systematic analyses (Delicado and Ramos, 2012). However, when morphological characters and molecular data are combined and considered together with ecological features and geographical patterns, a more clarified view of what is species is obtained for the group. Despite the complexity of studying this group, the high endemicity, in conjunction with extremely restricted distributions and habitats and limited dispersal capacities, make them useful candidates for correlating life-history traits with past geological events. Overall, this study demonstrates that comparison of both subgenera (as previously classified) reveals differences in geographical, ecological and evolutionary characteristics that exist between them.

Due to such differences and to avoid misunderstandings, in this discussion *P. (Pseudamnicola)* is sometimes referred to as *Pseudamnicola s. str.* and the *Pseudamnicola* genus as *Pseudamnicola s. l.*

PSEUDAMNICOLA (PSEUDAMNICOLA) IN THE IBERO-BALEARIC REGION: SYSTEMATICS AND BIOGEOGRAPHY

Species boundaries and phylogenetic reconstruction

Boeters (1988) redescribed the species *P. (P.) subproducta* and determined its distribution extended from the Iberian Peninsula to many localities in the Balearic Islands. Moreover, he found noteworthy variations in shell features and anatomical structures between Iberian populations and those from Majorca and Minorca (as well as differences between island populations). Such variability is observed on the illustrations but is not explained in the text of his paper (Boeters, 1988). In the section on *P. (P.) subproducta*, Boeters provided a general description for the species but mainly focused on characters related to the shell and female and male genitalia. Given such a characterization, all of the populations from the islands would be classified as *P. (P.) subproducta*; however, this can be explained by the fact that most of the characters status given by Boeters seem to be common within the subgenus and moreover shared by other *P. (Pseudamnicola)* species (see descriptions in Boeters, 1976; Ghamizi *et al.*, 1997; Szarowska *et al.*, 2006). For instance, shared characteristics include shell lengths between 3 to 3.5 mm, an elongate seminal receptacle and a slender penis that is two to four times longer than wide with a broad base and folding in its inner side of the proximal section (Boeters,

1988). Based on the description of each species, it seems that the relative dimensions of each organ, rather than the general shape, may discriminate between species. For example, the dimensions of the shell, the lengths of the bursa copulatrix, seminal receptacle or penis, and the nervous system concentration (RPG ratio), all vary among the studied species. Given these more specific differences, all of the Balearic populations originally classified by Boeters as belonging to *P. (P.) subproducta* actually correspond to four independent taxa, thus validating the species originally described by Glöer and Zettler (2007) and Altaba (2007) for these islands. Overall, this subgenus is morphologically represented in the Ibero-Balearic region by five species: *P. (P.) subproducta* in the Iberian Peninsula, *P. (P.) beckmanni*, *P. (P.) granjaensis* and *P. (P.) artanensis* in Majorca and *P. (P.) meloussensis* in Minorca. *P. (P.) gasulli* presents some anatomical peculiarities and its taxonomic status is discussed below.

Initial phylogenetic analyses of these six species recovered the existence of three main lineages grouping the Ibero-Balearic species of *P. (Pseudamnicola)*. The limited dispersal abilities of hydrobiids (Arconada and Ramos, 2003; Perez *et al.*, 2005; Hershler and Liu, 2008, Strong *et al.*, 2008) led to the hypothesis that the phylogeny of this group may be strongly related to the tectonic history of their habitats and therefore, for at least two or three clades, one would expect to find species unique to either the Iberian Peninsula or the islands of Majorca and Minorca. However, our phylogenetic reconstruction shows a different story. The species *P. (P.) subproducta* from the Iberian Peninsula and *P. (P.) meloussensis* from Minorca compose an unique clade, while the three species inhabiting Majorca Island constitute another clade and the species *P. (P.) gasulli* from the southeastern region of the Iberian Peninsula and Ibiza Island establishes a third clade (Figure 34).

It is also noteworthy that between the described morphospecies from Majorca Island the uncorrected p distances for the COI gene ranged between 0.5% and 1.7% (Appendix IV: Table 1), which is much lower than those determined between other species of hydrobiids (e.g. for the COI fragment, approximately 8% of genetic mean distance is recorded for *P. (Corrosella)* species in Delicado *et al.* (2012) and 3-5.5% for *Hydrobia* in Wilke *et al*., (2000a)). However, despite the low level of genetic divergence, differences on shell features and anatomy among *P. (P.) beckmanni*, *P. (P.) granjaensis* and *P. (P.) artanensis* are evident and sufficient to support the existence of these distinct species. This phenomenon of phenotypic diversity in molecularly closely-related species has also been observed in other evolutionary processes affecting snails, such as in case of (i) radiation (Perez *et al.*, 2005); (ii) dispersion associated with non-adaptive radiation (Gittenberger, 1991; Hershler ad Liu, 2008; Benke *et al.*, 2009); or (iii) recent speciation (Hershler *et al.*, 2007; Haase *et al.*, 2007a; Haase, 2008). Given that a radiation is less likely to give a pattern of isolation by distance, we conclude that the pattern of diversification of

Pseudamnicola s. str. on Majorca Island may be explained by a dispersion process followed by isolation and/or recent speciation (as shown in the chronogram in Figure 35). In support of a possible dispersion event, it is worth noting that species which are more similar molecularly, as in the case of *P. (P.) beckmanni* and *P. (P.) granjaensis*, also live in close proximity to each other compared to *P. (P.) artanensis*, which is more distant both genetically and geographically.

In contrast, the great genetic distance existing between *P. (P.) gasulli* and the rest of the studied *P. (Pseudamnicola)* species is also reflected in its characteristic morphology. Based on the most informative diagnostic characters available for hydrobiids, that is, the shell, genitalia, radula and nervous system (as in Davis *et al.*, 1992; Ramos *et al.*, 2000; Arconada and Ramos, 2006; Arconada *et al.*, 2007a, among others), all of the *P. (Pseudamnicola)* species occurring in the Ibero-Balearic region exhibit the morphological features associated with this subgenus with the exception of *P. (P.) gasulli.* The absence of a seminal receptacle and the presence of a strap-like penis with a narrow base make this species different from the rest of the known *P. (Pseudamnicola)* species. These autapomorphies together with its remarkable genetic distance may provide sufficient evidence to establish *P. (P.) gasulli* as belonging to a different genus. However, in addition to the six species described here, more taxa of both subgenera (*P. (Pseudamnicola)* and *P. (Corrosella)*) should be included in phylogenetic studies to fully support this hypothesis.

Biogeography and evolutionary patterns

The geological origins of the Balearic Islands are uncertain. Some authors suggest that this archipelago formed part of a continental macroplate, the Hercynian belt, which was attached to the eastern part of Iberia, and during the Oligocene (ca. 30-25 Ma) broke off and created several microplates (Corsica, Sardinia, Balearic Islands, Calabria, the Kabylies and the Betic-Rift plate) (Álvarez *et al.*, 1974; Giusti and Manganelli, 1984, Rosenbaum *et al.*, 2002). Under this theory, all of the Balearic Islands have a common origin. On the other hand, other authors propose that the Balearic Islands represent an extension of the external part of the Betic thrust, except for Minorca Island, which would have been associated with the Catalan Coastal Range (CCR). Based on this theory, Minorca, together with Corsica and Sardinia, would have composed a plate initially connected to the CCR, which during the middle Miocene (ca. 20 Ma) split with the aperture of Valencia Through (Fontboté *et al.*, 1990; Meléndez-Hevia, 2004). According to our age estimations, the split of the clade containing *P. (P.) gasulli* would be the only diversification process resulting as a consequence of continental flow, that is to say, allopatric or parapatric speciation. Therefore, the separation of the plates may have caused

fragmentation and subsequent isolation of the ancestral populations, leading them to evolve independently.

Overall, the cladogenetic events involving Majorcan and Minorcan species seem to have occurred later than the estimated origin of the Balearic Islands (for both theories), which suggest that the speciation process may have been related to transmarine colonization followed by vicariance. In order to inhabit the islands, dispersal had to play an important role, for instance, via aerial vectors (as in Gittenberger *et al.*, 2006; Kappes and Haase, 2012; Wada *et al.*, 2012) or temporary land bridges connecting the islands to the mainland, among other mechanisms. The first colonization, which was to Majorca Island, may have occurred in the range of 6.6 Ma to 2 Ma. The archipelago was connected to the mainland ca. 5.5 Ma, during the Messinian Salinity Crisis (MSC) (Carranza *et al.*, 2004; Fochetti *et al.*, 2009; Lázaro *et al.*, 2011), and thus, *P. (Pseudamnicola)* ancestral populations may have colonized the island through these connections. This expansion to the Balearic Islands during the MSC has also been observed for other invertebrate and vertebrate groups (Lalueza-Fox *et al.*, 2005; Lázaro *et al.*, 2011; Pérez-Losada *et al.*, 2011), most of which have limited dispersal capabilities. A second colonization event may have also occurred during this group's evolution. Around 3 Ma (between 4.5 Ma to 1.7 Ma), it appears that the common ancestor of the Iberian *P. (P.) subproducta* and the insular *P. (P.) meloussensis* colonized Minorca Island. It has also been postulated that the Balearic Islands may have once again been connected to the mainland during Plio-Pleistocene glaciations (Emig and Geistdoerfer, 2004; Bouzá-Ribot *et al.*, 2011). Accordingly, the expansion of *Pseudamnicola* to Minorca Island may have taken place through these connections.

These proposed events imply that there is not a common ancestor for Majorcan and Minorcan populations and that no evidence of dispersal or hydrobiid fauna interchange exists between them, despite both island having been connected to each other several times in the past (Brown *et al.*, 2008; Terrasa *et al.*, 2008; Pérez-Losada *et al.*, 2011). This pattern is similarly observed in two other hydrobiid species of the genus *Hydrobia* (Wilke *et al.*, 2000); thus, the same processes may have influenced the evolution of both *Hydrobia* and *Pseudamnicola*. The most recent diversification period was dated during the Pleistocene (1.8-0.1 Ma) and took place within Majorca Island. Mantel tests reveal that isolation by distance is the most likely explanation of the causes leading to speciation among the studied *P. (Pseudamnicola)* species. Likewise, the Pleistocene is characterized by oscillating glacial cycles (Veith *et al.*, 2003; Lalueza-Fox *et al.*, 2005) that could have caused shifts in freshwater flows. The impact of glaciations, especially on mountains such as where the Majorcan species mainly inhabit, could be greater, and this may have led to the isolation of freshwater habitats.

In conclusion, a double colonization scenario and subsequent isolation by vicariance, as well as other local speciation processes, may have been the main causes of *Pseudamnicola s. str.* species diversification in the Ibero-Balearic region.

PSEUDAMNICOLA (CORROSELLA) IN THE IBERO-BALEARIC REGION: SYSTEMATICS AND BIOGEOGRAPHY

Taxonomy and phylogenetic reconstruction

The discovery of the seven new species described along this thesis (six of them already published), besides elevating the formerly known diversity of *Pseudamnicola (Corrosella)* by more than 100%, reinforces the hypothesis of the Iberian Peninsula, and especially eastern Andalusia, as being one of the areas boasting the highest endemicity and diversity of Hydrobiidae in Europe (Arconada and Ramos, 2003).

From the present study, it can be concluded that these 12 species all exhibit generic morphological characters, except *P.? (C.) hydrobiopsis*. However, when the subgeneric features of *Corrosella* (as described by Boeters, 1970) were considered, one of the two diagnostic characters did not appear in these species. The problem is that the original diagnosis of *Corrosella* genus was provided both in French and German in the same paper (Boeters, 1970). According to the text in French, *Corrosella* was characterized by a bursa copulatrix at least twice as long as the accessory gland (= albumen gland + capsule gland in this work), whereas in the German text the bursa copulatrix was described as being as long as the accessory gland. Additionally, in the same paper (Boeters, 1970) and in French *Corrosella falkneri*, the type species of the new genus, was described as having a bursa copulatrix longer than the gland, which was in contradiction with the generic diagnosis both in French and German. The species here described and even *P. (C.) falkneri* show a bursa copulatrix shorter than the accessory gland (see Appendix III Table 5). All of this suggests that the relative lengths of these two organs should not be considered as diagnostic for *Corrosella*. Years later, *Corrosella* was included as a subgenus of *Pseudamnicola* (Boeters, 1984): the only difference between *Corrosella* and the nominal subgenus was the shape of the bursa, J-shaped in *Corrosella* and sac-shaped in *Pseudamnicola s. str.* Following the terminology of Hershler and Ponder (1998), the bursa copulatrix would vary from pyriform to cylindrical in *Corrosella* whereas it would be ovoid to pyriform in *Pseudamnicola s. str.* This study confirms the importance of the shape of the bursa copulatrix although it should not be used as the only diagnostic character to differentiate subgenera.

The morphological and morphometric data indicated that within the subgenus *Corrosella* there is no single presence/absence of characters to help with species identification. Species are differentiated by groups of quantitative characters in addition to those related to shell size and shape, and the relative positions of organs. The anatomical study revealed that the most informative characters are provided by the shell, female and male genitalia, radula and nervous system (as in Davis *et al.*, 1992; Ramos *et al.*, 2000; Arconada and Ramos, 2006; Arconada *et al.*, 2007a, among others).

On the other hand, analyzing phylogenetically these species, it is deduced that the subgenus *Corrosella* is composed of three well-supported lineages, which group the northern species in two clades and the southern ones in a third clade. Based on these results, the taxonomic status of each species is not only supported by the addition of more populations than just the type localities (as in Delicado *et al.*, 2012; Delicado and Ramos, 2012), but also by the other molecular markers analyzed. The distribution limits of some species were also redefined based on this work, for instance, *P. (C.) hinzi*, the only member of clade II, was only found in four localities in the Zaragoza and Teruel provinces, whilst some populations living in localities in the Burgos province (among them, Pozo Azul spring; Boeters, 1988), which were formerly attributed to *P. (C.) hinzi*, actually belong to *P. (C.) navasiana.* Hence, the distribution of *P. (C.) navasiana* is in fact much wider than previously reported (Boeters, 1988), whereas *P. (C.) hinzi* is restricted to two areas in the northern Iberian Peninsula.

Despite the fact that these species were well defined molecularly, our analysis did not completely resolve the phylogeny at basal levels or within clade III (Figure 76), where species inhabiting the southernmost points of the Betic Cordillera may have experienced a rapid speciation process.

Species of the subgenus *Corrosella* showed similar levels of divergence as those for other hydrobiid genera (based on uncorrected p-distances of mitochondrial genes). For instance, genetic divergences range between 1.1-13.1% for COI and 0.0-5.3% for 16S in the freshwater genus *Pyrgulopsis* (Liu and Hershler, 2005) and between 3-5.5% for COI in the brackish genus *Hydrobia* (Wilke *et al.*, 2000a). Between *P. (Corrosella)* species, mean divergences are between 5.6-12% for COI (Appendix IV: Table 1); in this case, the maximum values are similar with other genera, yet the minimum values are higher in *P. (Corrosella)*. Thus the limits of genetic variation within and between species differ from one genus to another either because of differences in mode and tempo of speciation between genera or because of the misidentification of species boundaries in hydrobiids due to the lack of valid morphological diagnostic characters. Sequence divergences of the nuclear 28S gene in *P. (Corrosella)* varied between 0-0.9%, which is slightly lower than the divergences observed in other rissooidean, such as *Bythinella* species, which have a

divergence of between 0.2-2% (Benke *et al.*, 2009).

Geological events and species diversification

The impact of tectonic activity on the freshwater habitats of *P. (Corrosella)* species influencing vicariant processes should be considered as a cause of diversification. The reconstruction discussed below takes into account the following issues: i) low dispersal ability of the group, ii) speciation and extinction and iii) the exclusion of passive dispersal (which has minor effects on the long-term life history of the group).

Both our phylogenetic analyses and molecular clock approach recovered three events of diversification that occurred during the evolution of this group approximately 10 Ma, 5 Ma and 2 Ma. Because these springsnails are freshwater organisms, their life-history traits may be strongly linked to the geographical evolution of the river basins. The present form of the Iberian river basins dates from the Quaternary (Vargas *et al.*, 1998); before this time, large endorheic lakes occupied the continental regions of the Iberian Peninsula. Given that the first radiation of *P. (Corrosella)* spp. was pre-Quaternary (approximately 10 Ma: 15.93 - 6.40 Ma based on coalescence analysis), the ancestor of the extant species would likely have diversified in such lakes or in their springs during the Miocene (specifically, from the middle Miocene to the middle Pliocene). Studies of intralacustrine speciation in ancient lakes, such as Lake Ohrid, have suggested horizontal and vertical barriers or gradients in lakes and their watersheds that cause differentiation (Albrecht and Wilke, 2008; Schreiber *et al.*, 2012). Therefore, similar speciation conditions could have impacted *Corrosella* speciation.

A second period of diversification occurred approximately 5 Ma (7.66 - 2.05 Ma), when many important geographic events took place in the Iberian Peninsula. In the northern area, up to Southern France, some populations were isolated probably due to the last uprising of the Pyrenees during the upper Miocene. The Pyrenees, acting as a geographical barrier, could have been the cause of vicariant events that affected northern *P. (Corrosella)* species, as well as other taxonomic groups, such as amphibians, reptiles and freshwater fishes (Barbadillo *et al.*, 1997; Griffiths, 2006; Perea *et al.*, 2010). This geological barrier may have led to the split between *P. (C.) astieri* and the group formed by *P. (C.) navasiana* and *P. (C.) hauffei*.

In the southern Iberian Peninsula, the high tectonic activity in the Alboran Sea area may have driven the diversification of species of clades IIIA and B, while species from clade I and II remained in the interior of the Iberian territory. Some studies reported that the microplates currently forming the Betic and Rif mountains

contacted southern Iberia 15 Ma (Rosembaum *et al.*, 2002; Weijermars, 1991), an event that may have caused the formation of clades IIIA and B. Furthermore, it has also been reported that during its initial continental stages the plate was occupied by multiple lacustrine basins oriented in a northeast to southwest direction (Aguirre, 2003) and that lacustrine sedimentation was predominant in this area (García-Aguilar and Martín, 2000). Therefore, the hydrobiids might have settled this plate environment. On the other hand, species diversification in the south (clade IIIB) could have involved allopatric speciation in the populations that inhabited the Alboran plate as it constituted a complex archipelago of islands during the lower Messinian (Alvinerie *et al.*, 1992; Barbadillo *et al.*, 1997). This vicariant process that occurred in the Betic-Rif Massif could have caused a certain degree of polymorphism, resulting in different genetic variants (Machordom and Doadrio, 2001), which, in our case, resulted in partially resolved phylogenetic relationships.

Given that fragmentation of habitats seems to have been a major factor causing the divergence of lineages, the configuration of the current river basins may have played an important role during the last diversification period of *Corrosella*. For example, the Iberian fluvial network was formed during the Plio-Pleistocene transition (Calvo *et al.*, 1993), and thus, could have influenced the last speciation events that occurred approximately 2 Ma (4.96 - 0.50 Ma).

Patterns and causes of diversification

Because *Corrosella* is a springsnail group, diversification processes may be strongly related to the evolution of their freshwater biotopes. Thus, factors responsible for speciation might be related to physical barriers (as discussed above) or ecological variables, or a combination of both. Based on our Mantel analyses, which tested the influence of some of these factors on the genetic pattern, only geographic distance had a positive significant correlation with the diversification of this group. This is reflected in the fact that species occurring in the northern Iberian Peninsula are genetically more related (in terms of genetic divergences) to species from the northeastern Betic Cordillera (i.e., *P. (C.) falkneri*, *P. (C.) manueli* and *P. (C.) bareai*) than with those from the southwestern Betic Cordillera (i.e., *P. (C.) iruritai*, *P. (C.) andalusica*, *P. (C.) ballestae* n. sp., *P. (C.) marisolae* and *P. (C.) luisi*) (see Appendix IV: Table 1). Although not all ecological variables were tested, our results suggest that the mechanism of diversification may be not related to adaptation to new environmental conditions but simply may be the result of habitat fragmentation and isolation. Gittenberger (1991) called this phenomenon non-adaptive radiation, and Wilke *et al.* (2010) demonstrated it with another springsnail genus, *Bythinella*, which has similar types of habitats as *P. (Corrosella)*, but in different distribution areas. The diversification of both genera seems to fulfill the

criteria for considering it a non-adaptive radiation: i) no clear niche differentiation, ii) a low degree of phenotypical variation and iii) species evolution usually under allopatric conditions. We highlight that even if *P. (Corrosella)* species occasionally live in sympatry with other hydrobiids in the study area, we never found any of the *P. (Corrosella)* species living together.

Apart of the geological processes aforementioned, climatic conditions could have led *P. (Corrosella)* spp. to have different patterns of speciation in the different regions. On one hand, during the Pleistocene glaciations the group may have suffered a period of extinction, especially in northern and central Iberia. In these regions, the subgenus is mainly represented by *P. (C.) navasiana*, possibly due to the recolonization of empty niche space left from depleted spring fauna. On the other hand, the intensive tectonic activity at the Betic Cordillera during the Miocene may be the main mechanism driving differentiation, thus leading to a higher level of speciation in the southern region. Furthermore, a higher precipitation index during the Miocene in the northern part of Spain compared to the southern part (Jiménez-Moreno *et al.*, 2010) could have played a role in habitat connectivity. Studies with pollen spectra suggest that, during the Miocene to Pliocene, the south of Spain was characterized as having strong seasonality, including drought periods that lasted between seven and nine months (Jiménez-Moreno *et al.*, 2010). Thus, water stress and climatic oscillations may have led to fragmented and isolated habitats, which resulted in a higher level of speciation in the south (clade III).

In contrast to the generally restricted, homogeneous habitat pattern that characterizes this subgenus, *P. (C.) navasiana* (part of the northern clade I) has a wide distribution range and high ecological plasticity, being able to adapt to a wide variety of habitats, including ones at different altitudes, river basins and localities with varying levels of temperature and conductivity (see Appendix II). Moreover, taking into account that no species sympatry is known within *Corrosella*, the wide distribution of *P. (C.) navasiana*, to some extent, prevents the expansion of other congeneric species. A prolonged stay in the presumptive ancestral distribution area (the corresponding endorheic lake), with a climate that enhanced the connection between different water masses in the northeastern Iberian Peninsula, might in part account for *P. (C.) navasiana* particularities and consequently the lower level of species diversity in the northern Iberian Peninsula. This idea can be linked to the hypothesis of niche conservatism, that was first proposed by Wiens (2004) and Kozak and Wiens (2006), stating that allopatric speciation may be produced by a combination of phylogenetic niche conservatism and climate oscillations, at least in temperate clades. The original concept postulates that the narrower the niche is, the more likely habitats are to be fragmented resulting in higher species richness. Studies in other groups, such as salamanders (Kozak and Wiens, 2010) and

zopherine beetles (Baselga *et al.*, 2011), have also suggested a high level of niche conservatism.

In the case of *Corrosella*, where a remarkable difference in distribution range and species richness exists between the northern and southern clades, a correlation between habitat features and molecular divergence is not observed. In fact, the evolutionary history of *Corrosella* is better explained by a non-adaptive radiation that can be related to different paleogeographic events, from the Miocene to the Pleistocene, coupled with climatic episodes that determined the potential connectivity between areas of varying degrees of isolation. Only *P. (C.) navasiana* does not follow the general rules of the group, and thus, it may be the key to understanding the primary causes that determined the whole history of the group.

THE GENUS *PSEUDAMNICOLA*: TAXONOMY, PHYLOGENETIC PATTERNS AND EVOLUTIONARY HISTORY

As previously mentioned, *Pseudamnicola* was first proposed by Paulucci (1878) to differentiate between European and American *Amnicola* species, characterizing both groups mainly by their conchological features. Nearly a century later, Boeters (1970) studied the genus anatomically and based on differences in female genitalia defined a new genus, *Corrosella*, designating *Corrosella falkneri* Boeters, 1970 as its type species (Boeters, 1970). The main difference between *Corrosella* and *Pseudamnicola s. str.* was in the shape of the bursa copulatrix: J-shaped in *Corrosella* and sac-shaped in *Pseudamnicola s. str.* However, given the lack of other diagnostic characters for identifying each genus, Boeters concluded that *Corrosella* should be a subgenus within *Pseudamnicola* (Boeters, 1984). This exemplification demonstrates the necessity of further studies in order to produce more exhaustive morphological and anatomical descriptions as well as well-supported, consistent phylogenies. Hydrobiidae *s. str.*, and concretely *Pseudamnicola*, have weakly sculptured shells and small and simple anatomical structures (Arconada and Ramos, 2003; Bichain *et al.*, 2007; Strong *et al.*, 2008), making it difficult to establish clear species boundaries. To clarify the systematic status of the genus *Pseudamnicola*, and its subgenera, our work is based on a tripartite approach: anatomical descriptions, morphometric studies and molecular phylogenetics.

Review of morphological characters

In order to define and taxonomically classify the *Pseudamnicola* species occurring in the Iberian Peninsula, the "standardized" characters for hydrobiids as defined by Hershler and Ponder (1998), as well as other characters such as the nervous system, were examined. Overall, our anatomical results indicate that, within the genus, quantitative characters and estimates of their variability at the species level are essential for species identification and delimitation. Indeed, each of the described (or redescribed) species in this work is not characterized by only a single trait, but rather a combination of defining, quantitative characters (as in Wilke *et al.*, 2002). Although all of the species studied here exhibit morphological diagnostic characters previously attributed to *Pseudamnicola s. l.* (see Boeters, 1988; Ghamizi *et al.*, 1997; Delicado *et al.*, 2012), the boundaries of the genus may be better defined in light of the work presented in this study.

All in all, from the comparison of anatomical structures, we classify the interspecific characters in three categories (in order of taxonomical value without taking into account the diagnostic characters for the family): i) characters taxonomically non-informative as synapomorphies shared by all species of *Pseudamnicola s. l.* and even present in its sister subfamily Hydrobiinae (Hershler and Davis, 1980; Boeters, 1988; Wilke *et al.*, 2002; Arconada and Ramos, 2003; Szarowska, 2006), such as the aperture of the shell ovate, operculum features, one pair of basal cusps in the central radular tooth, pigment on head and foot, the presence of ctenidium and osphradium, the presence of pigment on a coiled renal oviduct, a bean-shaped prostate gland, a pigmented nervous system, the length of cerebral ganglia and the presence of a gastric caecum; ii) homologous characters derived from a recent common ancestor (apomorphies) and therefore applicable for defining species and their relationships, i.e. SL/SW ratio (as in Hershler, 1989; Ramos *et al.*, 2000; Arconada and Ramos, 2001; 2006; Arconada *et al.*, 2007a), the shape and length of the seminal receptacle and length of the bursa copulatrix and bursal duct (as in Ramos *et al.*, 2000; Arconada and Ramos, 2001; Arconada *et al.*, 2007a), extension of the pigment on the renal oviduct, shape and dimensions of the penis (Hershler, 1989; Szarowska, 2006; Arconada *et al.*, 2007b) and dimensions of the prostate gland (as in Arconada *et al.*, 2007a) and iii) homoplasic characters shared by two genetically independent individuals and hence, not valid for establishing phylogenetic relationships among species, i.e. the microsculpture of the protoconch and the number of gill filaments (Hershler and Ponder, 1998; Arconada *et al.*, 2007b), the number of cusps in radular teeth (Szarowska, 2006), the number of teeth rows of the radula, the attachment of the penis base to the head, the length of the seminal receptacle duct and the RPG ratio (contrary to the hypothesis of Fretter and Graham (1962) who proposed that more elongated connectives are a primitive state).

By and large, the second category shows the most valid traits for differentiating between *Pseudamnicola s. l.* species and for reconstructing their phylogenetic relationships. In particular, characters related to female genitalia seem very important taxonomically, not only for the genus examined in this work, but also for many hydrobiid taxa (Davis and Carney, 1973). In several hydrobioids studies (Davis *et al.*, 1992; Szarowska, 2006; Wilke *et al.*, 2013), female genital features accounted for between 25-50% of the most discriminate anatomical characters, followed by the male reproductive system and the digestive system. Boeters (1984) also asserted that female genitalia play an important role in the diagnosis of both subgenera, which is consistent with our results, though with some caveats. Boeters (1984) claimed that *P. (Corrosella)* possesses a J-shaped bursa copulatrix, whereas *P. (Pseudamnicola)* has a sack-shaped form. Based on the terminology of Hershler and Ponder (1998), most of the species of both subgenera bear a pyriform bursa copulatrix with differences in length (longer in *P. (Corrosella)*). Nevertheless, the female character that is genus specific for its presence, shape and position (Boeters, 1988; Ramos *et al.*, 2000; Arconada and Ramos, 2001; Szarowska, 2006), and moreover has resulted to be different within *Pseudamnicola* species, is the seminal receptacle. Three different morphologies have been found for this structure: absent in *P. (P.) gasulli*, whereas in the other species it is present and elongate, but longer in *P. (Pseudamnicola)* than in *P. (Corrosella)*. The loss of the seminal receptacle does not imply major alterations in the physiology of the organism inasmuch as the function of this organ (i.e. sperm storage) can be replaced by the albumen gland or renal oviduct (Ponder, 1988; Ramos *et al.*, 2000; Szarowska, 2006). Actually, some females of *P. (P.) gasulli* presented a thickening in their renal oviduct, which may indicate the presence of this function. The absence of the seminal receptacle is a primitive character in gastropods (Ponder and Lindberg, 2000); however, this character state seems to occur rarely within Hydrobiidae *s. str.* (for instance *Tarraconia*, Ramos *et al.*, 2000), suggesting that an evolutionary trace may not be established with this data.

Within male genitalia, the shape and dimensions of the prostate gland and penis seem to be important variables for differentiating between sister species as well as among the three *Pseudamnicola* lineages (as shown in the discriminant analysis). The penis is the most informative male genital organ and its characteristics are usually constant within a species and between groups of species (Arconada and Ramos, 2006). Within the genus, three different morphologies have been observed for this copulatory organ: gradually tapering in *P. (Corrosella)*, triangular in *P. (Pseudamnicola)* and strap-like in *P. (P.) gasulli*. On the other hand, although the prostate gland is not considered a phylogenetic character in hydrobiids (Hershler and Ponder, 1998), our morphometric analysis has revealed significant differences in its dimensions between the subgenera and the species *P. (P.) gasulli*.

Although derived characters tend to be more appropriate for establishing phylogenetic relationships (Hennig, 1966) and defining species, homoplasies possess an evolutionary importance from an adaptive point of view. One of the morphological structures that show a high level of homoplasy is the ctenidium and its number of gill filaments. The presence of a well-developed ctenidium in hydrobiids is an ancestral character, and its reduction seems to occur independently in some lineages. On the one hand, the loss of gill filaments has been attributed to the adaptation to amphibious life (Fretter, 1948; Ponder *et al.*, 1989) and on the other, to the reduction of body size (Fretter and Graham, 1994; Hershler and Ponder, 1998; Arconada and Ramos, 2001). All of the *Pseudamnicola* species studied here live submerged under water. Despite occupying environments with differences in conductivity and altitude (i.e. variables that affect oxygen levels), these species do not present substantial differences in gill filament numbers. Furthermore, the *Pseudamnicola* genus co-occurs with other hydrobiid genera which lack ctenidiums entirely, for instance *Chondrobasis levantina* Arconada and Ramos, 2001, *Boetersiella sturmi* (Rosenhauer, 1856), *Boetersiella davisi* Arconada and Ramos, 2001 or *Josefus aitanica* Arconada and Ramos, 2006. Hence, given that species with and without well-developed ctenidium can inhabit the same locality, habitat features may have not affected the evolution of this trait. Regarding body size, it is noteworthy that the smallest species described here, *P. (C.) falkneri*, has about half the number of gill filaments than *P. (C.) luisi*, which is one of the largest species. Thus, our results indicate that reduction of body size may influence ctenidium development.

Likewise, convergence is suspected in the evolution of radula and shell features. Radula features are useful in some cases for characterizing species, though it is not a good phylogenetic marker within *Pseudamnicola s. l.* since this group shows high levels of homoplasy. This may be because the radula is part of the digestive system and adaptative processes to different types of foods or substrates may have influenced its morphology (Davis and McKee, 1989; Hawkins *et al*., 1989). The shell is a key element in malacological taxonomy; however, in the case of *Pseudamnicola s. l.*, cryptic characteristics are more pronounced within than between lineages, making it difficult to differentiate species, especially in those involved in the radiation that occurred in southern Spain. Variation in gastropod shell shape is a matter of genetics but in combination with environmental factors (Urdy *et al.*, 2010), convergent processes may consequently play an important role in the evolution of this trait (Haase, 2008). Nevertheless, in hydrobiids, shell features do not seem largely influenced by the environment as this character is synapomorphic in many clades within this family (Szarowska, 2006), despite differences in habitat conditions. For example, an ovate-conic shell shape characterizes both Pseudamnicolinae and the brackish subfamily Hydrobiinae. Therefore, genetics may be a stronger component on shell evolution. In any case, a

small variation in shell size has been found among the three groups of *Pseudamnicola* (see descriptions and discriminant analysis), and such differences may be due to environmental biotic and abiotic factors, such as parasitism, competition, food availability, salinity or water temperature (Wilke *et al.*, 2002; Wilke and Falniowsky, 2001; Haase, 2003; Haase, 2008). However, determination of the actual causes of shell variation is beyond the scope of this work.

Overall, although some morphologic characters are taxonomically informative for differentiating hydrobiid taxa, and particularly *Pseudamnicola* species, we affirm the absolute need of combining morphology with the information provided by molecular and ecological approaches. In other words, the application of integrative taxonomy is required in order to improve knowledge about the species (Haase *et al.*, 2007b; Padial *et al.*, 2010). The following sections of this study aim to apply this approach.

Phylogenetic framework and biogeography

The application of molecular tools in the systematic analysis of *Pseudamnicola* has revealed the existence of three main lineages within the genus, corresponding to the two subgenera previously described plus the species *P. (P.) gasulli*. From this molecular study, we conclude that the observed morphological differences existing between the two subgenera have a phylogenetic signal and moreover, that the anatomical differences recorded for *P. (P.) gasulli* reflect a different origin of this species with respect to other *P. (Pseudamnicola)* species. The combined mitochondrial and nuclear phylogeny reasonably supports each of the clades; however, the relationships among them still remain unclear.

Although well supported as monophyletic groups, *P. (Pseudamnicola)* and *P. (Corrosella)* display different evolutionary patterns (reflected in Figure 80). In *P. (Pseudamnicola)*, splitting events appear more recent and with less-supported branches, whereas in *P. (Corrosella)*, the branches are longer and more robust, which is a possible sign of a more ancestral and gradual speciation process within this group. One reasonable explanation for the different topologies may be because *P. (Corrosella)* species present more restricted distribution ranges and inhabit springs and headwaters of streams, which often act as isolated habitats (Wilke *et al.*, 2010). Thus, these locations may constrain gene flow between populations (Brändle *et al.*, 2005) and increase the degree of endemicity. In contrast, *P. (Pseudamnicola)* species and *P. (P.) gasulli* are brackish-water species and occur in brackish streams, lagoons and low river stages where the ecological conditions are less restrictive and the waters remain connected. Moreover, such locations are more expose to the presence of birds and fishes than springs (Haase, 2008), which may favor dispersion

via vectors. In any case, these two habitat prototypes are likely associated with two different dispersal models, directly influencing their phylogenetic topologies.

The average pairwise divergence in the COI partition between species is 1.5 times greater in *P. (Corrosella)* than in *P. (Pseudamnicola)*. Sequence differences (measured as uncorrected pairwise distances) between spring snails species of *P. (Corrosella)* ranged between 5.3% to 12% (with an average of 9%), which is similar to ranges described for other springsnail genera, such as *Bythinella* (1.5-13.4% in Bichain *et al.*, 2007), and *Floridobia*, *Marstonia* and *Pyrgulopsis* (0.5-6.1%, 1.0-8.5% and 2.8-11.2%, respectively in Hershler *et al.*, 2003). Alternatively, genetic divergences for *P. (Pseudamnicola)* species are an average of 6.7% (ranging between 0.5% to 10%), which falls between the estimated 9% for *P. (Corrosella)* and 4.5% for the brackish genus *Hydrobia* (Wilke *et al.*, 2000a). To a limited extent, this gradient of genetic divergences may be due to the type of environment (freshwater *vs.* brackish water) occupied by these three groups (see previous section).

In general, *P. (Corrosella)* species occur in isolated habitats, present a clear biogeographical pattern and genetically distinct species. On the other hand, the geographical patterns of *P. (Pseudamnicola)* are not as explicit. A possible reason could be because the number of samples examined is more limited, and thus, the entire distribution range of this subgenus has not been covered. It would be interesting to first, extend the study in the sampled regions, and second, to genetically examine the species described from Morocco and Algeria (Ghamizi *et al.*, 1997; Glöer *et al.*, 2010) in order to investigate whether the current distribution of the group is from stochastic or tectonic processes. In any case, as no clear biogeographic pattern is present among the studied populations, an alternative explanation for their distribution pattern may be long distance colonization followed by isolation. As the principal aim of this work is to study the relationships between species and their biogeographical distribution, no population genetic level analyses have been performed, thus we cannot hypothesize which distribution model the populations of this subgenus have followed. Further research at the population level and over a larger area of study is required.

Nevertheless, phylogenetic analysis of the group does reveal the existence of three well-supported lineages within the genus. The anatomy and morphometrics reflect substantial differences between the subgenera and highlighted *P. (P.) gasulli* as a different entity bearing evidences of divergence. Taking all these results into account, we suggest that the three lineages may correspond to three different genera, raising *Corrosella* to the category of genus once again (as in Boeters, 1970) and removing *P. (P.) gasulli* from *P. (Pseudamnicola)*, thus making itself a new genus. In addition to the anatomical and morphological characteristics that sufficiently distinguish them as different genera, the genetic divergences that exist between them

(uncorrected distances ranged between 11.1% to 14.3% for COI and between 6.7% to 8.4% for 16S) are similar to those reported between other genera. For instance, between genera belonging to the sister subfamily Hydrobiinae (based on phylogenies in Wilke *et al.*, 2001; Szarowska, 2006; Wilke *et al.*, 2013), i.e. *Adriohydrobia*, *Hydrobia*, *Peringia* and *Ventrosia*, molecular distances range between 10.4% to 14.8% for COI and 2.3% to 5.8% for 16S (uncorrected pairwise distances, Wilke, 2003). Therefore, the subfamily Pseudamnicolinae would be composed of three genera, one freshwater and two medium-brackish. Thus, within the current phylogeny for hydrobiids (Wilke *et al.*, 2013), these two subfamilies are very interesting from an evolutionary perspective because they may represent a transition between two different environments.

Evolutionary history: tempo and mode of diversification

All of the phylogenetic inferences performed in this work have suggested the division of *Pseudamnicola s. l.* into three lineages, which may correspond to three different genera. Observing their patterns of diversification, each of these genera is likely to have experienced different evolutionary processes in space and time. Based on the available taxonomic sampling performed for each lineage and the coalescence analysis (Figure 83) using an evolutionary rate previously cited for hydrobiids, we estimate that the split leading to these three lineages likely occurred during the upper-middle Miocene (28-17 Ma). Although not all of the described *Pseudamnicola s. l.* species have been included and not all of the areas of its distribution range have been sampled in this work, our current results suggest that the Iberian Peninsula has played an important role in the diversification of the group as it is, to date, the only region in which all three proposed genera inhabit. Thus, given the more restrictive distribution pattern of *Corrosella* and the distribution pattern of the new genus (composed of *P. (P.) gasulli*) with respect to that of *Pseudamnicola*, their evolution may be a result of a peripatric (allopatric) speciation that occurred in the Iberian Peninsula. During the Oligocene and Miocene (between 35 Ma and 5.33 Ma), the Iberian Peninsula suffered a compressive period with the loss as well as adhesion of several continental fragments (Meléndez-Hevia, 2004) in which most of the Iberian ranges originated, thereby affecting the region's hydrological system. The creation of these physical barriers may have caused an isolation process followed by vicariance by which the geographical range of the last common ancestor of the three genera was fragmented in a relatively short period of time, thus leading to the separate lineages. However, it seems that some populations not only remained isolated but also evolved and adapted to new habitat conditions in a mountainous environment (as in the case of the genus *Corrosella*). It is noteworthy that our results revealed significant differences in habitat features (altitude and conductivity) between genera, which may be a consequence of an adaptive process.

Therefore, this may be a case in which there is some implicit degree of natural selection in allopatric speciation, as postulated by Wright (1931).

With respect to *Corrosella* species, their current systematic and distribution could be the result of vicariant and climatic events that occurred in the Iberian Peninsula during the Miocene, or of a process of isolation by distance (Wright, 1943) (also supported by our Mantel test), or a combination of both. The Miocene was a crucial period for the Iberian Peninsula because of several geological processes related to Alpine orogeny that critically affected the diversification and dispersion of many species of plants and animals (Joger *et al.*, 2007; Miguel *et al.*, 2007; Pardo *et al.*, 2008). The Iberian Peninsula suffered a compressive period in which the current mountain ranges were created. These palaeogeographical episodes led to fragmented habitats of *Corrosella* and as a consequence, to allopatric speciation within the group, which increased its species richness and changed dispersion patterns. For instance, the second and last uprising of the Pyrenees in the North (Barbadillo *et al.*, 1997; Meléndez-Hevia, 2004) may have influenced the split between *C. astieri* and *C. navasiana* - *C. hauffei*. In addition, the creation of the Betic Cordillera and the active plate tectonics in the southern and eastern regions of the Alboran Sea (approximately 10 Ma, according to Rosenbaum *et al.*, 2002) may have been one of the reasons the radiation involved southern *Corrosella* species. Finally, the creation of the Iberian hydrological network during the Quaternary (Vargas *et al.*, 1998) may have led to vicariant processes that influenced the most recent splitting of *Corrosella* species.

Furthermore, species of *Pseudamnicola s. str.* have been found on the Mediterranean basin mainland, peninsulas and islands, yet the species *P. subproducta* only inhabits the Iberian Peninsula. Therefore, our results point towards the hypothesis that the creation and diversification of *Pseudamnicola s. str.* did not originate in the Iberian Peninsula. The inclusion of more populations from different Mediterranean localities in future analyses will likely identify the evolutionary scenario of the group. In any case, the coalescence analysis performed with the available data shows a rapid cladogenetic event whose period does not mismatch any of the hypotheses discussed above, as such event seems to have been posterior to the geological origin of the western Mediterranean region and anterior to Plio-Pleistocenic glaciations. Thus, the tree topologies and estimated divergences identify the Messinian salinity crisis (between 5.96-5.33 Ma, according to Krijgsman *et al.* (1999), or 6.5/6.6-5.3 Ma, according to Aguirre (2003)) as the main driver for the diversification of *Pseudamnicola s. str.*

Based on both the divergence time estimate and the current distribution patterns of *Pseudamnicola s. str.*, we deduce that such a cladogenetic event is more likely to have occurred by a process of colonization of several Mediterranean regions during the MSC followed by isolation, rather than by a vicariant event

produced by fragmentation of a larger habitat. The poor dispersal capability of freshwater snails (they require permanent water courses, although they may survive desiccation for several days) (Jensen *et al.*, 1996; Haase *et al.*, 2010), and their inability to cross marine water masses make the hypothesis of colonization less plausible. However, the estimated periods of diversification of *Pseudamnicola* species are subsequent to when the Mediterranean peninsulas and islands were formed and therefore, it is likely that the populations arrived after that event. Two alternative scenarios may also explain the colonization process: i) via land bridges connecting several plates, for instance between Sardinia, Tunisia, Sicily and Italy (Rosenbaum *et al.*, 2002; Goes *et al.*, 2004) or between the Balearic Islands and the Iberian Peninsula (Carranza *et al.*, 2004; Fochetti *et al.*, 2009; Lázaro *et al.*, 2011), both of which were established during the Messinian (6-5 Ma) and Plio-Pleistocenic glaciations or ii) through passive extra-aquatic dispersion via water birds. This dispersal mechanism has been reported as the most feasible explanation for the distribution pattern of other hydrobioids, especially for brackish genera (Wilke and Davis, 2000; Liu *et al.*, 2003; Haase *et al.*, 2010; Kappes and Haase, 2012). Indeed, this hypothesis has recently been confirmed by Wada *et al.* (2012), who demonstrated that gastropods can pass through and survive the digestive system of birds and can even subsequently produce offspring, making predation by birds a possible method of dispersal. Kappes and Haase (2012) proposed several active and passive dispersal mechanisms for freshwater mollusks. However, in this case, no geographic criterion exists and multiple tectonic plates are involved, therefore long-distance dispersal via birds may be more likely hypothesis to explain this fact.

OUTPUTS AND FUTURE WORK

In this thesis, a revision of the springsnail genus *Pseudamnicola s. l.* in the Iberian Peninsula and Balearic Islands has been presented. This work is far from being a closed investigation: more and new questions have arisen from the progress of this study, which will probably be addressed in future works.

One of the main outputs of this thesis is related to the taxonomic status of the genus. Using an integrative approach, combining morphology, morphometrics and molecular studies, one new genus has been identified and a subgenus, once considered a genus, has been validated as a genus again. In addition, seven new species have been identified under this approach and six out of them have been published as a result of this thesis (Figure 85). The descriptions of the two new genera are the subject of a manuscript in preparation.

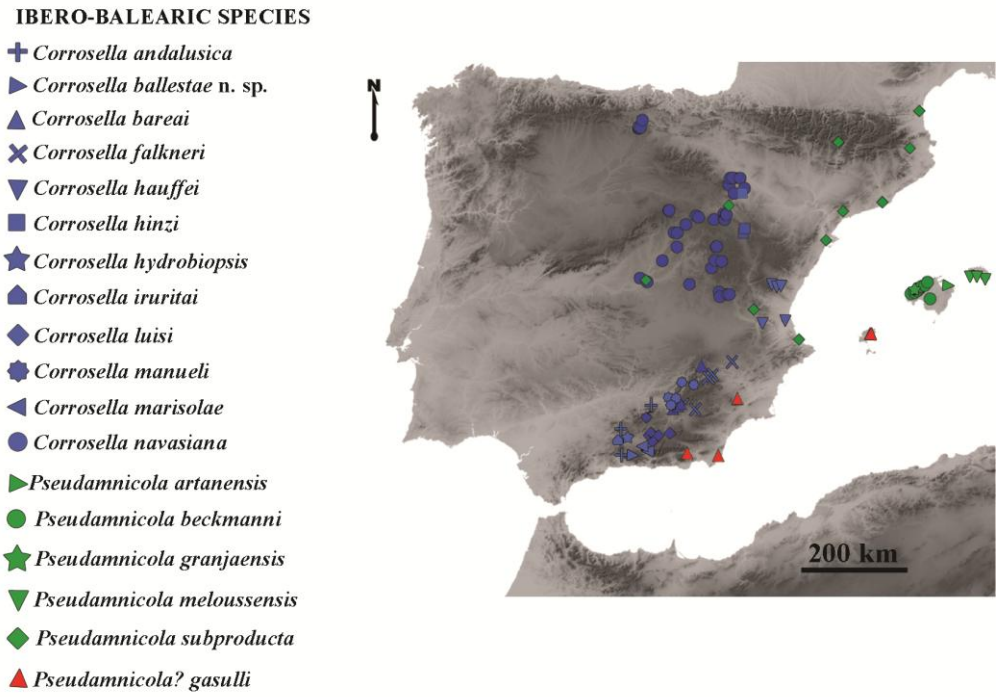


Figure 85. Ibero-Balearic geographic map displaying the schematic map showing the distribution of each species examined in this thesis. Each color refers to a genus and each symbol to a species.

These three genera would compose the subfamily Pseudamnicolinae whose sister groups would be the subfamilies Hydrobiinae and Pyrgulinae (according to Wilke *et al.*, 2013, see Figure 86). Therefore, future research should focus on extending the sampling area beyond the Ibero-Balearic region in order to: i) ascertain the endemic character of the two new genera revealed in this study and thereby testing the hypothesis that genera diverged due to mountainous barriers; ii) study the biogeography of the genus *Pseudamnicola s. str.* more thoroughly and thereby verifying whether the current distribution is a result of a vicariant process caused by plate tectonics in the Mediterranean basin or by dispersal processes; and iii) discover possible new genera within the subfamily.

More generally, for a better understanding of the systematics and evolution of the family Hydrobiidae, the molecular clock should be calibrated. As several species have been described from both sides of the Gibraltar Strait and the opening of this strait is well calibrated, a reasonable rate could be obtained through the study of the relationships of those species. Once calculated, the phylogeny published by Wilke *et al.* (2013) could be calibrated and thus, the causes of diversification discussed in this thesis evaluated. Furthermore, given the close relationship between the subfamilies Pseudamnicolinae and the brackish Hydrobiinae, the direction of the

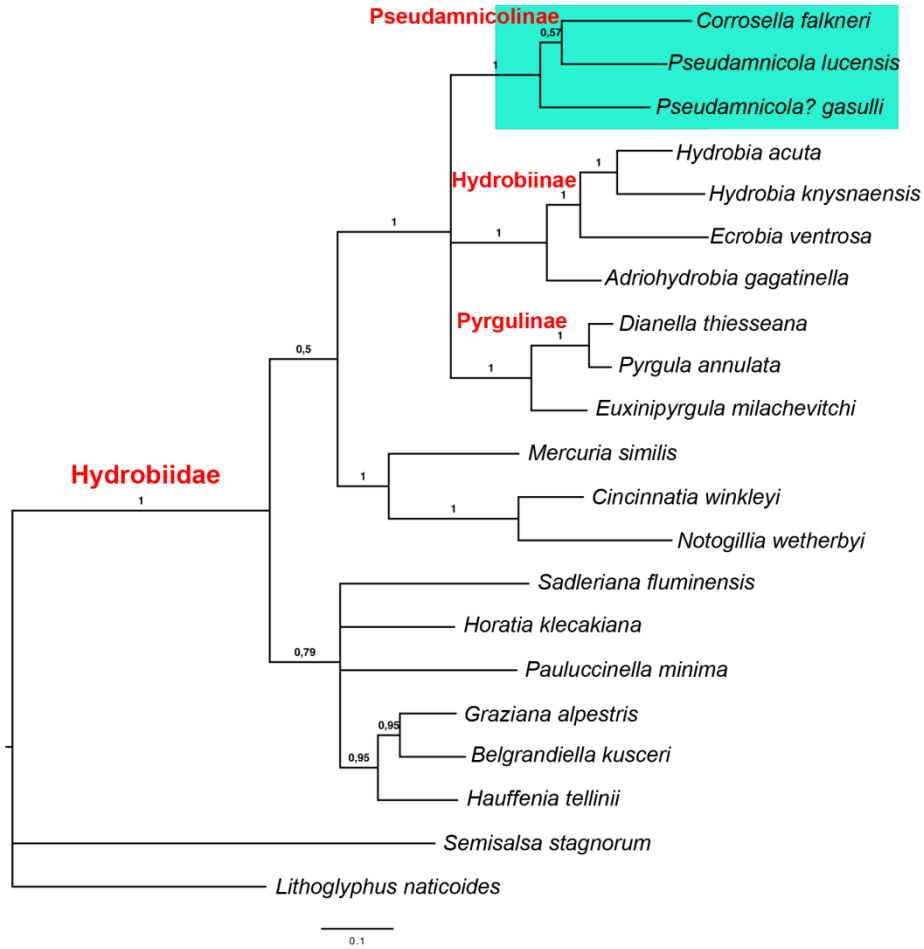


Figure 86. Bayesian inference of Hydrobiidae taxa based on the three fragments COI, 16S and 18S. This preliminary analysis has been performed in order to assess the relationships of *Pseudamnicola s. str.* as well as *Corrosella* and the new genus within the phylogeny of the family. Sequences taken from the phylogeny published in Wilke *et al.*, 2013 and implemented with the two new genera here discovered. BPP's are provided over branches.

colonization may be polarized, that is, from freshwater environments to brackish ones or vice versa. Likewise, it would also be interesting to examine the morphological character evolution and the effects on morphology and anatomy that such an environmental transition may have had in these subfamilies. The overall objective to be gained by studies such as the one presented in this thesis is to advance the study of this fascinating group so that their current habitats and populations will be preserved. If we do not fulfill this objective, hydrobiids, as well as many other species, inhabiting freshwater and brackish ecosystems may be affected, which would produce an irreversible loss of biodiversity and make this project and future investigations closed and limited.

CONCLUSIONES

The overall conclusions reached from the results presented in this thesis are the following:

1. According to the morphological and molecular results, *Pseudamnicola s. l.* is composed of three genera: *Pseudamnicola s. str*., *Corrosella* (originally described as a genus, but later established as a subgenus), and a new genus comprised of the species *P. (P.) gasulli*, with the latter two genera likely endemic to the Iberian Peninsula. The tempo and mode of diversification of these three groups seem to be different, which may be related to habitat. Whereas *Corrosella* spp. occur in isolated springs and headwaters of streams in mountainous regions, species belonging to the other two genera inhabit brackish streams, lagoons and low river stages where waters remain connected, thus allowing gene flow between populations.

2. The diversity of *Pseudamnicola s. l.* in the Ibero-Balearic region was previously underestimated. The number of species within the study area for this group is now 18, seven of which are based on the results of this work, thereby increasing the total number of known species in this area by approximately 40%. All are endemic to this region with restricted distributions, except the species *C. navasiana*, which extends further into northeastern Spain, and *P. subproducta*, which has a wide but patchy distribution.

3. Confirming the conclusions of previous works, the most valid diagnostic characters distinguishing *Pseudamnicola s. l.* species seem to be: the SL/SW ratio, the shape and length of the seminal receptacle, the length of bursa copulatrix and bursal duct, the extension of pigment on the renal oviduct, the shape and dimensions of the penis and the dimensions of the prostate gland. Characters related to the digestive system and pallial organs were insufficient for differentiating between taxa.

4. Although the mitochondrial fragments 16S rRNA and COI proved useful for characterizing species, some of the phylogenetic relationships remain unresolved in the single-tree topologies. Combining these partitions with the nuclear 28S rRNA gene was beneficial for resolving some of the polytomies; though, those that are likely to be a result of a rapid speciation remain unclear.

5. According to morphological and molecular analyses, the populations described for Majorca and Minorca, which were previously classified as *P. subproducta*, in actuality, constitute four independent species, all belonging to *Pseudamnicola s. str*. The Minorcan species *P. meloussensis* is clustered

with the Iberian species *P. subproducta,* while the three Majorcan species comprise a unique clade.

6. The fifth species inhabiting the Balearic Islands, previously identified as *P. (P.) gasulli,* is anatomically and genetically distinct from those belonging to the other two genera. This species forms an independent phylogenetic clade distinct from *Pseudamnicola* and *Corrosella*, and anatomical characters, such as the absence of a seminal receptacle, the presence of a strape-like penis or a smaller prostate gland can also distinguish it as a different genus.

7. Dating studies show that the separation of the Balearic Islands from the continent likely occurred prior to the split between the Iberian and Balearic species. Hence, the occurrence of the Balearic species does not seem to be a consequence of the geological origin of the Balearic Islands, but rather may be related to two subsequent transmarine colonizations followed by vicariance.

8. The newly reestablished genus *Corrosella* is composed of 12 Iberian species and *C. astieri* from southern France. All of them are phylogenetically clustered in three well-supported lineages, which group the northern species in two clades and the southern ones in a third clade (though this clade is not monophyletic in coalescent analyses).

9. The phylogenetic relationships among the three *Corrosella* lineages remain unresolved at basal levels. Furthermore, the lack of resolution within the third clade may indicate that the species inhabiting the southernmost areas of the Betic Cordillera experienced a rapid speciation process.

10. Statistical tests reveal that diversification patterns of *Pseudamnicola s. l.* species were strongly related to habitat fragmentation rather than to environmental conditions. Taking this into account, the diversification of the Iberian *Corrosella* spp. was probably related to vicariant and climatic events that occurred in the Iberian Peninsula during the Miocene (especially during the Messinian Salinity Crisis), accompanied by a process of isolation by distance.

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APÉNDICES

Apéndice I: Listado de especies de *Pseudamnicola* distribuidas globalmente.

Europa

P. (C.) andalusica Delicado, Machordom y Ramos, 2012. Península Ibérica.

P. (C.) astieri (Dupuy, 1851). Francia continental.

P. (C.) bareai Delicado, Machordom y Ramos, 2012. Península Ibérica.

P. (C.) falkneri (Boeters, 1970). Península Ibérica.

P. (C.) hauffei Delicado y Ramos, 2012. Península Ibérica.

P. (C.) hinzi Boeters, 1986. Península Ibérica.

P. (C.) hydrobiopsis Boeters, 1999. Península Ibérica.

P. (C.) iruritai Delicado, Machordom y Ramos, 2012. Península Ibérica.

P. (C.) luisi Boeters, 1984. Península Ibérica.

P. (C.) manueli Delicado, Machordom y Ramos, 2012. Península Ibérica.

P. (C.) marisolae Delicado, Machordom y Ramos, 2012. Península Ibérica.

P. (C.) navasiana (Fagot, 1907). Península Ibérica.

P. (P.) artanensis Altaba, 2007. Islas Baleares.

P. (P.) bacescui Grossu, 1986. Rumania.

P. (P.) beckmanni Glöer y Zettler, 2007. Islas Baleares.

P. (P.) brachia (Westerlund, 1886). Creta.

P. (P.) chamasensis Boeters, 2000. Francia continental.

P. (P.) chia (E. von Martens, 1889). Islas del norte del mar Egeo.

P. (P.) confinis (Brancsik, 1897). Bosnia.

P. (P.) conovula (Frauenfeld, 1863). Croacia.

P. (P.) cyrniacus (J. Mabille, 1869). Córcega.

P. (P.) dobrogica (Grossu, 1986). Rumanía.

P. (P.) exilis (Frauenfeld, 1863). Grecia continental.

P. (P.) gasulli Boeters, 1981. Península Ibérica.

P. (P.) granjaensis Glöer y Zettler, 2007. Islas Baleares.

P. (P.) leontina Grossu, 1986. Rumanía.

P. (P.) lucensis (Issel, 1866). Italia continental.

P. (P.) macrostoma macrostoma (Kuster, 1853). Islas Cícladas, Grecia continental e islas del norte del mar Egeo.

P. (P.) macrotoma negropontina (Clessin, 1878). Grecia.

P. (P.) malickyi Schütt, 1980. Chipre.

P. (P.) meloussensis Altaba, 2007. Islas Baleares.

P. (P.) moussonii (Calcara, 1841). Italia continental, Malta, Cerdeña y Sicilia.

P. (P.) orsinii (Kuster, 1852). Italia continental y Sicilia.

P. (P.) penchinati (Bourguignat, 1870). Rumanía.

P. (P.) pieperi Schütt, 1980. Islas Dosecanesas de Grecia.

P. (P.) pisolinus (Paladilhe, 1876). Francia continental.

P. (P.) pyrenaicus Boeters y Falkner, 2009. Francia continental.

P. (P.) razelmiana Grossu, 1986. Rumanía.

P. (P.) sciaccaensis Glöer y Beckmann, 2007. Sicilia.

P. (P.) subproducta (Paladilhe, 1869). Península ibérica.

P. (P.) troglobia Bole, 1961. Bosnia y Croacia.

P. (P.) virescens (Kluster, 1853). Macedonia.

Norte de África

P. (P.) algeriensis Glöer, Bouzid y Boeters, 2010. Argelia.

P. (P.) boucheti Glöer, Bouzid y Boeters, 2010. Argelia.

P. (P.) calmensis Glöer, Bouzid y Boeters, 2010. Argelia.

P. (P.) chabii Glöer, Bouzid y Boeters, 2010. Argelia.

P. (P.) dupotetiana (Forbes, 1838). Marruecos.

P. (P.) fineti Glöer, Bouzid y Boeters, 2010. Argelia.

P. (P.) gerhardfalkneri Glöer, Bouzid y Boeters, 2010. Argelia.

P. (P.) ghamizii Glöer, Bouzid y Boeters, 2010. Argelia.

P. (P.) linae Glöer, Bouzid y Boeters, 2010. Argelia.

P. (P.) meluzzii Boeters, 1976. Túnez.

P. (P.) pallaryi Ghamizi, Vala y Bouka, 1997. Marruecos.

P. (P.) rouagi Glöer, Bouzid y Boeters, 2010. Argelia.

P. sinaica Pallary, 1901. Egipto

P. abxazica Badzoshvili, 1979. Georgia.

P. bchirikvarensis Badzoshvili, 1979. Georgia.

P. bilgini Shütt y Sesen, 1993. Turquía.

P. buxinensis Yu and Zhang, 1982. China.

P. fluxinensis Yu, 1987. China.

P. geldiayana Shütt y Bilguin, 1970. Turquía.

P. georgievi Glöer y Pešić, 2012. Irán.

P. intranodosa Shütt y Sesen, 1993. Turquía.

P. kotschyi v. (Frauenfeld, 1863). Irán.

P. liaoxiensis Yu, 1987. China.

P. likharevi Izzatullaev, 1973. Tajikistan.

P. narzikulovi Izzatullaev, 1972. Tajikistan.

P. optima Yu, 1977. China.

P. pavlovskii Izzatullaev, 1973. Tajikistan.

P. raddei Boettger, 1889. Irán.

P. saboori Glöer y Pešić, 2009. Irán.

P. taoyuanensis Youlou, 1978. China.

P. zagrosensis Glöer y Pešić, 2009. Irán.

Apéndice II: Localidades muestreadas en la región Íbero-Balear y sur de Francia con presencia de *Pseudamnicola*.

Códigos de los campos: Alt.: Altitud de la localidad; T: Temperatura del agua; C: Conductividad del agua. N. D. indica que no se obtuvo ningún dato para ese campo. Las localidades cuyos individuos han sido estudiados genéticamente se muestran señalados con (*).

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
P. (C.) astierii (*)	Source d' Argens, Brue-Auriac, Francia	21/06/2010	31T 0735019/4820889	D.D.	286	15,0	0,75	Etanol 70% y absoluto
P. (C.) andalusica (*)	Fuente de La Salud, Albanchez de Mágina, Jaén, España (localidad tipo)	10/05/2009 16/02/2010	30S 0459006/4181398	I.B.	N. D.	N. D.	N. D.	Etanol 70% y absoluto
(*)	Fuete Eduardo, Alcaucín, Málaga, España	03/12/2008	30S 04008/4085	J.M. B.	767	N. D.	N. D.	Etanol 70% y 96%
(*)	Fuente El Piojo, Almedinilla, Córdoba, España	05/01/2009	30S 0404049/4143497	J.M. B.	997	N. D.	N. D.	Etanol 96%
	Río Turvilla, Canillas de Albaida, Málaga, España	05/12/2008	30S 0414592/1040904	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
	Fuente Morellana, Luque, Córdoba, España	01/2009	30S 0390679/4154251	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
	Fuente Pilar, Cabra, Córdoba, España	14/01/2009	30S 0379367/4151133	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
P. (C.) ballestae n. sp. (*)	Fuente en Jatar, Granada, España (localidad tipo)	06/2009 23/01/2010	30S 0418451/4087781	I.B.	990	N. D.	N. D.	Etanol 70%, 96% y 100%
P. (C.) bareai (*)	Fuente en la Ermita de las Santas, Collados de la Sagra, Granada, España (localidad tipo)	06/06/2006 13/10/2007 21/05/2008	30S 0542294/4201835	J.M. B. D.D.	1345	15,0	0,78	Etanol 70% y congelado -80ºC
(*)	Agüerillo, Castril, Granada, España	21/05/2008	30S 0517351/4186925	J.M. B.	1274	N. D.	N. D.	Etanol 96%
(*)	El Laude, Castril, Granada, España	21/05/2008	30S 0517139/4187270	J.M. B.	1274	N. D.	N. D.	Etanol 96%
(*)	Fuente Nuevas, Castril, Granada, España	21/05/2008	30S 0512019/4181258	J.M. B.	1123	N. D.	N. D.	Etanol 96%

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
(*)	Siete Fuentes, Cuenca, Jaén, España	12/10/2007	30S 0502639/4176601	D.D.	1030	N. D.	N. D.	Etanol 70% y congelado -80ºC
(*)	Fuente La Plata, Riopar, Albacete, España	30/03/2008	30S 0559052/4260118	D.D.	949	15,0	0,43	Etanol 70% y congelado -80ºC
	Fuente Alfahuara, María, Almería, España	05/04/2010	30S 0566986/4173648	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
P. (C.) falkneri (*)	Fuente La Armada, Orce, Granada, España	21/05/2008 30/10/2008	30S 0546272/4176139	J.M. B.	890	N. D.	N. D.	Etanol 70% y 96%
(*)	Fuente Palo, Orce, Granada, España	21/05/2008 30/10/2008	30S 0545712/4175851	J.M. B.	911	N. D.	N. D.	Etanol 70% y 96%
	Fuente Zarza, Orce, Granada, España	21/05/2008	30S 0544907/4176049	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
	Fuente La Pi, Orce, Granada, España	21/05/2008	30S 0544632/4175976	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
	Fuente Las Mimbreras, Orce, Granada, España	21/05/2008	30S 0545875/4175182	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
	Fuente Tubos, Castril, Granada, España	21/05/2008	30S 0520451/4185742	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
	Fuente en Pontezuela, Granada, España	21/05/2008	30S 0519553/4184431	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
	Fuente en Castril, Granada, España	26/08/2006	30SWH2087	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
(*)	Dos Caños spring, Castril, Granada, España	13/10/2008	30S 0520478/4185772	D.D.	1077	21,0	0,57	Etanol 70% y congelado -80ºC
(*)	Fuente La Errá, La Dehesa, Albacete, España	30/03/2008	30S 0572400/4242458	D.D.	793	19,0	0,50	Etanol 70% y congelado -80ºC
	Arroyo en Letur, Albacete, España	12/10/1994	30 S 0578584/4245895	N.M., D.M.	N. D.	N, D.	N. D.	Etanol 70%
	Fuente García en Cordovilla, Albacete, España	18/05/1997	30 S 0619287/4269179	N.M., D.M.	N. D.	N. D.	N. D.	Etanol 70%
	Oria, Almería, España	12/2009	30S 0570250/450420	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
P. (C.) hauffei (*)	Fuente de los Nogales, Benafer, Castellón, España (localidad tipo)	26/05/1998 19/03/2009	30S 0707446/4422810	E.R D.D., C.N.	548	12,0	N. D.	Etanol 70% y congelado -80ºC
	Fuente Agadín, Benafer, Castellón, España	19/03/2009	30S 0707132/4423868	D.D., C.N.	575	15,0	N. D.	Etanol 70% y congelado -80ºC
	Acequia en Navajas, Castellón, España	07/03/1990	N. D.	R.A., D.M. y J.M. R.	N. D.	N. D.	N. D.	Etanol 70%
(*)	Fuente del Curso, Navajas, Castellón, España	25/05/1998	30S 0713817/4416740	B.A.	391	N. D.	N. D.	Etanol 70% y congelado -80ºC
	Fuente de la Peña, Navajas, Castellón, España	07/03/1990	N. D.	R.A., D.M. y J.M. R.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente de la Esperanza, Navajas, Castellón, España	07/03/1990	N. D.	R.A., D.M. y J.M. R.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente del Prado, Viver, Castellón, España	19/03/2009	30S 0703907/4423503	D.D., C.N.	646	16,0	N. D.	Etanol 70% y congelado -80ºC
(*)	Fuente de San Miguel, Viver, Castellón, España	25/05/1998 19/03/2009	30S 0704181/4422501	B. A. D.D., C.N.	662	15,0	N. D.	Etanol 70%
	Acequia de la fuente de San Miguel, Viver, Castellón, España	19/03/2009	30S 0704181/4422501	D.D., C.N.	662	15,0	N. D.	Etanol 70% y congelado -80ºC
	Font Nova, Benifaio, Valencia, España	26/05/1998	30S 0721924/4351560	B.A.	N. D.	N. D.	N. D.	Etanol 70%
	Cortés de Pallás, Valencia, España	28/05/1998	30S 0677580/4345921	B.A.	N. D.	N. D.	N. D.	Etanol 70%
P. (C.) hinzi (*)	Balsa de Vargas, Borja, Zaragoza, España	07/10/2009	30T 0620319/4631116	R.M A.	453	19,0	0,80	Etanol 70% y absoluto
(*)	Fuente Cazuelas, Borja, Zaragoza, España	14/07/2009	30T 0620448/4631055	R.M A.	458	19,2	0,80	Etanol 70% y absoluto
(*)	Fuente en el parque fluvial de Calamocha, Teruel, España	10/12/2009	30T 0643260/4531871	R.M A.	874	17,3	0,95	Etanol 70% y absoluto

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
(*)	Fuente del Prado, Caminreal, Teruel, España	10/12/2009	30T 0640448/4522829	R.M A.	916	15,4	0,83	Etanol 70%
P. (C.) iruritai (*)	Fuente de Don Pedro, Loja, Granada, España (localidad tipo)	05/09/2007 20/04/2009 16/02/2010	30S 0399460/4115074	J.M. B. D.D., C.N. J.M. B.	505	15,0	0,61	Etanol 70%, 96% y absoluto
P. (C.) luisi (*)	Fuente de La Gitana, La Peza, Granada, España	27/09/1989 25/03/1998 12/05/2007 12/03/2009	30S 047415/412475	E. R. B.A D.D. J.M. B.	1026	N. D.	N. D.	Etanol 70% y absoluto y congelado -80ºC
(*)	Fuente Grande, Dieza, Granada, España	23/04/1992 25/03/1998 22/08/2006 07/05/2008	30S 04592/41308	D.M. B.A. J.M. B.	1575	N. D.	N. D.	Etanol 70% y 96%
(*)	Arroyo Polvorista, Quéntar, Granada, España	27/10/2006	30S 0509318/ 4194330	J.M. B.	1316	N. D.	N. D.	Etanol 96%
(*)	Fuente de La Teja, Sierra de Huétor, Granada, España	30/09/2008	30S 0455050/4124207	J.M. B.	1354	N. D.	N. D.	Etanol 96%
	Arroyo de La Grea, Maitena, Granada, España	27/09/1989	30S 04616/41127	E.R., D.M. y J.M. R.	N. D.	N. D.	N. D.	Etanol 70%
	Acequia en la Sierra de Huétor, Granada, España	22/08/2006	30 S 495820 4129400	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
	Manantial Fardés en Diezma, Sierra Harana, Granada, España	12/10/1992	30S 04592/41308	E.R., D.M.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente Grande en Prado Negro, Granada, España	26/05/2006	30 S 0450000/4160000	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
P. (C.) manueli (*)	Arroyo de la Garganta, Nava de San Pedro, Jaén, España (localidad tipo)	01/05/1990 12/10/2007	30S 0509318/4194330	N.M., D.M. D.D.	1429	12,0	0,50	Etanol 70% y 90% y congelado -80ºC

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
	Fuente de La Ponderosa, Hinojares, Jaén, España	30/04/1990	30S 0501944/4179988	N.M., D.M.	N. D.	N. D.	N. D.	Etanol 70%
(*)	Fuente El Céfano, La Iruela, Jaén, España	23/03/1998 14/10/2007	30S 0502253/4197687	B.A. D.D.	774	15,0	0,54	Etanol 70% y congelado -80ºC
	Acequia del Molino, La Iruela, Jaén, España	30/04/1990	30S 0500538/4197129	N.M., D.M.	N. D.	N. D.	N. D.	Etanol 70%
	Acequia en Finca Rechita, La Iruela, Jaén, España	23/03/1998	30S 050028/419810	B.A.	N. D.	N. D.	N. D.	Etanol 70%
(*)	Arroyo El Valle, La Iruela, Jaén, España	14/10/2007	30S 0504010/4196581	D.D.	1209	15,0	0,51	Etanol 70% y congelado -80ºC
	Hotel en Sierra de Cazorla, La Iruela, Jaén, España	30/04/1990	30S 05005/41969	N.M., D.M.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente en Prados de la Presa, Jaén, España	24/03/1998	30S 520352/4230312	B.A.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente La Mata en Mata Bejid, Jaén, España	24/03/1998	30S 04553/41721	B.A.	N. D.	N. D.	N. D.	Etanol 70%
	Arroyo en la Toba, Jaén, España	24/03/1998	30S 0539412/4226322	B.A.	N. D.	N. D.	N. D.	Etanol 70%
	Arroyo en el Museo de la caza, Cazorla, Jaén, España	01/05/1990	30S 0500538/4197129	N.M., D.M.	N. D.	N. D.	N. D.	Etanol 70%
(*)	Acequia San Isidro, Cazorla, Jaén, España	20/05/2009	30S 0499837/4195755	J.M B., I.B.	791	N. D.	N. D.	Etanol 96%
P. (C.) marisolae (*)	Fuente Pilar del Mono, Dúrcal, Granada, España (localidad tipo)	25/09/1989 17/10/1989 15/10/1990 08/02/1992 27/03/1998 22/09/2008	30S 0449218/4095030	E.R., D.M., N.M., B.A., J.M. R., J.M. B.	786	N. D.	N. D.	Etanol 70% y 90%
(*)	Fuente Palmones, Padul, Granada, España	28/02/2007	30S 0445770/ 4097570	J.M. B.	808	N. D.	N. D.	Etanol 96%

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
	Padul, Granada, España	20/09/2006	30 S 444359 4097921	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
	Fuente Grande, Alfácar, Granada, España	18/10/2008	30S 045991/4122546	J.M. B.	N. D.	N. D.	N. D.	Etanol 96%
P. (C.) navasiana (*)	Manantial de Fonnueva, Bulbuente, Zaragoza, España (localidad tipo)	27/10/1995 21/08/2005 01/05/2007 11/05/2009	30T 0634067/4630860	B.A., E.R., J.M. R., R.M. A., D.D.	N. D.	N. D.	N. D.	Etanol 70% y absoluto y congelado -80ºC
	Acequia del manantial de Fonnueva, Bulbuente, Zaragoza, España	01/05/2007	30T 0614407/4630677	R.M. A., D.D.	N. D.	N. D.	N. D.	Etanol 70%
	Ojos de Cimballa, Zaragoza, España	24/02/2005	N. D.	R.M. A.	N. D.	N. D.	N. D.	Etanol 70%
	Cimballa, Zaragoza, España	18/10/1995 19/10/1995	N. D.	B.A., E.R., J.M. R.	N. D.	15,6	0,95	Etanol 70% y congelado -80ºC
	Acequia en Cimballa, Zaragoza, España	27/05/2010	30T 0603437/4549548	R.M. A.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente del Molino Nuevo, Cimballa, Zaragoza, España	27/05/2010	30T 0603296/4549600	R.M. A.	N. D.	18,3	0,53	Etanol 70%
	Manantial Huerta bajera, Cimballa, Zaragoza, España	25/05/2010 27/05/2010	30T 0602551/4550534	R.M. A.	N. D.	18,7	0,52	Etanol 70%
	Río Mesa, Zaragoza, España	19/10/1995	N. D.	B.A., E.R., J.M. R.	N. D.	12,1	N. D.	Etanol 70% y congelado -80ºC
	Fuente Magdalena, Ginel river, Zaragoza	26/10/2011	N. D.	R.M. A.	N. D.	N. D.	N. D.	Material en seco
	Fuente Lagüen, Aranda del Moncayo, Zaragoza	26/10/2011	N. D.	R.M. A.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente en Monterde, Zaragoza, España	19/10/1995	30 T 606086 4558832	B.A., E.R., J.M. R.	N. D.	N. D.	N. D.	Etanol 70% y congelado -80ºC
	Canal en Maleján, Zaragoza, España	27/10/1995	30 T 619189 4631589	B.A., E.R., J.M. R.	N. D.	N. D.	N. D.	Etanol 70% y congelado -80ºC

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
	Mesones, Zaragoza, España	27/10/1995	N. D.	B.A., E.R., J.M. R.	N. D.	N. D.	N. D.	Etanol 70%
(*)	Fuente en Mesones, Zaragoza, España	10/02/2009	30T 0622209/4600711	R.M. A.	N. D.	14,4	1,15	Etanol 70%
(*)	Lago del Espejo, Nuévalos, Zaragoza, España	27/05/2010	30T 0601752/4561056	R.M. A.	N. D.	18,2	0,62	Etanol 70% y absoluto
	Fuente de los Contrabandistas, Zaragoza, España	25/05/2010	30T 0547488/4553271	R.M. A.	N. D.	15,6	0,36	Etanol 70%
	Fuente de San Roque, Monterde, Zaragoza, España	27/05/2010	30T 0606975/4558852	R.M. A.	N. D.	17,6	0,49	Etanol 70% y absoluto
	Ojos de Pontil, Zaragoza, España	27/05/2010	30T 0642618/4611010	R.M. A.	N. D.	22,8	1,21	Material en seco
	Fuente del Tope, Talamantes, Zaragoza, España	02/12/2009	30T 0612142/4618	R.M. A.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente en Bolea, Huesca, España	22/11/2011	N. D.	R.M. A.	N. D.	N. D.	N. D.	Etanol 70% y absoluto
	Fuente El Suso, Deza, Soria, España	26/10/2011	30T 0581941/4591074	R.M. A.	889	18,6	N. D.	Etanol 70%
(*)	Fuente Tinte, Medinaceli, Soria, España	25/05/2010	30T 0548191/4556254	R.M. A.	N. D.	15,5	0,27	Etanol 70% y 96%
(*)	Fuente en Arbujuelo, Soria, España	25/05/2010	30T 0552244/4553716	R.M. A.	N. D.	16,4	0,44	Etanol 70% y absoluto
(*)	Pozo Azul, Covanera, Burgos, España	01/05/2008	30T 0434832/4731870	D.D.	704	13,0	0,50	Etanol 70% y congelado
(*)	Fuente La Toba, Tubilla del agua, Burgos, España	01/05/2008 07/03/2009	30T 0434228/4728442	D.D.	794	13,0	0,45	Etanol 70% y congelado
(*)	Arroyo Valdeménez, Sedano, Burgos, España	01/05/2008	30T 0434461/4729589	D.D.	750	12,0	0,39	Etanol 70% y congelado
(*)	Arroyo en Tubilleja, Burgos, España	11/05/1997 03/05/2008	30T 0441661/4744676	B.A., D.D.	624	14,6	0,50	Etanol 70% y congelado

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
(*)	Arroyo en Valtubilla, Sedano, Burgos, España	01/05/2008	30T 0438406/4730753	D.D.	742	13,0	0,38	Etanol 70% y congelado
(*)	Fuente de las Canalejas, Somolinos, Guadalajara, España	05/04/2008	30T 0494082/4567372	D.D.	1259	13,0	0,32	Etanol 70% y congelado -80ºC
	Laguna de Somolinos, Guadalajara, España	30/04/1989 01/04/1990 13/04/1990 17/04/1990	30T 04942/45676	D.M., N.M.	1238	11,0	0,29	Etanol 70%
	Río Mesa, cerca de Maranchón, Guadalajara, España	26/02/2011	30T 577568/4539331	R.M. A.	N. D.	13,5	1,08	Etanol 70% y absoluto
	Río Mesa, cerca de Anquela del Ducado, Guadalajara, España	26/02/2011	N. D.	R.M. A.	N. D.	13,8	1,24	Etanol 70% y absoluto
	Manantial del río Mesa, Selas, Gadalajara, España	26/02/2011	30T 575396/4534020	R.M. A.	N. D.	13,2	0,42	Etanol 70% y absoluto
	Fuente de la Charquilla, Selas, Guadalajara, España	26/02/2011	30T 575379/4534030	R.M. A.	N. D.	12,9	0,38	Etanol 70% y absoluto
	Canal entre el manantial del río Mesa y la fuente de la Charquilla, Guadalajara, España	26/02/2011	30T 575378/4534044	R.M. A.	N. D.	12,8	0,39	Etanol 70% y absoluto
	Río Mesa, cerca de Selas, Guadalajara, España	26/02/2011	30T 0575305/4534074	R.M. A.	N. D.	13,2	0,42	Etanol 70% y absoluto
	Fuente Umbría, Casas de San Galindo, Guadalajara, España	25/08/1991	30T 0503511/4524512	D.M., N.M.	N. D.	N. D.	N. D.	Etanol 70%
	Mochales, Guadalajara, España	19/10/1995	30T 0582791/4549802	B.A., E.R., J.M.R.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente en Peralejos de las truchas, Guadalajara, España	05/04/2008	30T 0587855/4496132	D.D.	1187	12,5	0,52	Etanol 70% y congelado -80ºC
	Río Tajo, Peralejos de las truchas, Guadalajara, España	22/07/1990 06/04/2008	30T 0587855/4496132	D.M., N.M. D.D.	1187	12,5	0,57	Etanol 70% y congelado -80ºC

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
(*)	Nacimiento del río Bornova, Guadalajara, España	05/04/2008	30T 0493728/4567290	D.D.	1238	12,5	0,44	Etanol 70% y congelado -80ºC
(*)	Río Dulce, Cabrera, Guadalajara, España	06/09/1989 06/04/2008	30T 0527218/4539658	D.M. D.D.	953	N. D.	N. D.	Etanol 70% y congelado -80ºC
	Fuente en Valfermoso de las Monjas, Guadalajara, España	18/08/1991 05/04/2008	30T 0509833/4523763	D.M., N.M. D.D.	900	16,0	0,55	Etanol 70% y congelado -80ºC
	Balsa en Irueste, Guadalajara, España	03/08/2008	30T 0509282/4495656	R.M. A.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente y balsa en Almodóvar, Cuenca, España	09/03/1990	30S 0594176/4397936	R.A., D.M., y J.M. R.	993	19,0	0,52	Etanol 70%
	Fuente del Roble, Yémeda, Cuenca, España	09/03/1990 17/04/1990 07/11/1996	30S 0609305/4402269	R.A., D.M., J.M. R., B.A., S.J.	868	16,5	1,50	Etanol 70%
	Fuente, arroyo y canal en Uña, Cuenca, España	15/05/1990	30T 0586854/4453076	D.M., N.M.	1150	N. D.	N. D.	Etanol 70%
	Arroyo del río Huécar, Cuenca, España	21/04/1996	N. D.	J.B.	1076	N. D.	N. D.	Etanol 70%
	Río Huécar, Cuenca, España	21/04/1996	N. D.	J.B.	N. D.	N. D.	N. D.	Etanol 70%
	Fuente cerca Palomera, Cuenca, España	21/04/1996	30S 0534217/4422100	J.B.	1076	N. D.	N. D.	Etanol 70%
	Fuente en Cueva del Fraile, Cuenca, España	21/04/1996	3TWK7838	J.B.	N. D.	N. D.	N. D.	Etanol 70%
	Tragacete, Cuenca, España	01/06/1996	30T 0597707/4467364	J.B.	1283	N. D.	N. D.	Etanol 70%
	Fuente del Rincón, Monteagudo de las Nátivas, Cuenca, España	07/11/1996	30S 05927/44071	B.A., S.J.	1007	N. D.	N. D.	Etanol 70%
	Fuente del Piojo, Cardete, Cuenca, España	08/11/1996	30S 06128/44028	B.A., S.J.	963	N. D.	N. D.	Etanol 70%
	Villalba de la Sierra, Cuenca, España	08/11/1998	30T 0577600/4454270	M.A. R.	999	N. D.	N. D.	Etanol 70%

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
(*)	Fuente de la Tía Perra, El Hosquillo, Cuenca, España	09/03/2008	30S 0584468/4469395	D.D.	1361	12,0	0,45	Etanol 70% y congelado -80ºC
	Olmeda de las Fuentes, Madrid, España	14/02/2010	30T 0481228/4468725	O.S.	N. D.	N. D.	N. D.	Etanol 70% y congelado -80ºC
(*)	Fuente de La Errá, La Dehesa, Albacete, España	30/03/2008	30S 0572400/4242458	D.D.	771	19,0	0,50	Etanol 70% y congelado -80ºC
(*)	Acequia en Borox, Toledo, España	09/06/2007	30S 0437550/4435450	D.D.	580	17,0	3,74	Etanol 70% y congelado -80ºC
	Balsa en Nuevo Borox, Toledo, España	10/06/2007	N. D.	D.D.	N. D.	20,0	2,07	Etanol 70% y congelado -80ºC
(*)	Fuente María, Ontígola, Toledo, España	21/10/2007 21/11/2009	30S 0451163/4427676	D.D., B.A., C.N.	595	18,0	3,20	Etanol 70% y absoluto y congelado -80ºC
P. (P.) artanensis (*)	Fuente cerca de la Ermita de Betlem, Artá, Mallorca, (localidad tipo)	18/04/2008	31S 0527040/4398503	D.D.	273	16,0	0,84	Etanol 70% y absoluto
P. (P.) beckmanni (*)	Fuente del Rentador, Deyá, Mallorca	16/04/2008	31S 0469953/4399665	D.D.	141	17,0	0,45	Etanol 70% y absoluto
(*)	Fuente en Valdemossa, Mallorca	16/04/2008	31S 0467929/4395647	D.D.	N.D.	18,0	1,44	Etanol 70% y absoluto
	Fuente Son Moragues (finca privada), Valdemossa, Mallorca	16/04/2008	N.D.	M.A. R.	N.D.	N.D.	N.D.	Etanol 96%
	Font d S'Aigueta , carretera de Valdemossa a Palma, Mallorca	04/12/2006 11/11/2006	31S 0468285/4394480	M.A. R. B.A.	267	N.D.	N.D.	Etanol 70% y congelado -80ºC
	Acequia en Sóller, Mallorca	12/11/2006 04/12/2006 17/04/2008	31S 0475313/4401226	B.A. M.A. R. D.D.	N.D.	22,0	0,50	Etanol 70% y absoluto
	Fuente de S'Artiga, Sóller, Mallorca	17/04/2008	31S 0473100/4398182	D.D.	479	14,5	0,50	Etanol 70% y absoluto

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
	Font des Noguer, Fornalutx, Mallorca	17/04/2008	31S 0482825/4404398	D.D.	29	13,0	0,55	Etanol 70% y absoluto
	Arroyo de bajada en la Granja de Esporles, Mallorca	19/4/2008	31S 0462128/4391039	D.D.	285	16,0	0,60	Etanol 70% y absoluto
	Manantial en la Granja de Esporles, Mallorca	01/02/2008	N.D.	M.A. R.	N.D.	N.D.	N.D.	Etanol 96%
	Acequia en Lluch, Mallorca	17/08/1990	N.D.	R.A., M.C.	N.D.	N.D.	N.D.	Etanol 70%
	Fuente en Estellenchs, Mallorca	11/11/2006	N.D.	B.A.	N.D.	N.D.	N.D.	Etanol 70%
(*)	Fuente cerca de Randa, Mallorca	09/10/2010	N.D.	M.A. R.	N.D.	N.D.	N.D.	Etanol 70% y 90%
(*)	Jardines de Alfabia, Mallorca	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Etanol 70%
P. (P.) granjaensis (*)	Pilón de salida del Palacio de la Granja de Esporles, Mallorca	19/04/2008	31S 0462143/4391091	D.D.	255	17,0	0,63	Etanol 70% y absoluto
P. (P.) meloussensis (*)	Fuente en cala Macarella, Menorca	08/12/2007	31S 0580075/4421464	D.D., B.A., J.Q.	4	19,0	1,30	Etanol 70% y absoluto
(*)	Font de la Reina, Son Saura, Menorca	08/12/2007	31S 0576881/4420917	D.D., B.A., J.Q.	14	18,0	1,40	Etanol 70% y absoluto
(*)	Torrente en el barranco de Son Boter, Menorca	08/12/2007	31S 0589092/4419398	D.D., B.A., J.Q.	66	16	1,18	Etanol 70% y absoluto
(*)	Barranco de Sen Penyes, Es Canutells, Menorca	09/12/2007	31S 0599908/4412149	D.D., B.A., J.Q.	11	16	2,57	Etanol 70% y absoluto
P. (P.) subproducta (*)	Font Estramar, Salse le Château, Francia	18/06/10	31T 0496513/4745147	D.D.	10	17	7,35	Etanol 70% y absoluto
(*)	Ullals de Baltasar, Amposta, Tarragona	30/03/1990 13/03/1999 16/07/2007	31T 0296036/4505021	J.B., D.M., R.A., B.A., D.D.	10	20	2,12	Etanol 70% y absoluto y congelado -80ºC
(*)	Les Borges del Camp, Tarragona	2012	N.D.	M.V.	N.D.	N.D.	N.D.	Etanol absoluto

Especie	Localidad	Fecha de captura	UTM	Colector	Alt. (m)	T (ºC)	C (mS)	Método de fijación
(*)	Desagüe de la laguna de Ontígola, Madrid	16/04/1993 21/10/2007 21/11/2009	30S 0448699/4429964	M.A. R., N.M., D.M. D.D., B.A., C.N.	545	17	3,64	Etanol 70% y congelado -80ºC
(*)	Fuente Flores, Requena, Valencia	08/03/2008	30S 0661128/4372781	D.D.	660	15	0,81	Etanol 70% y congelado -80ºC
	Font d' Izquerdo o dels Tramussos, Oliva, Valencia	27/05/1998	30SYJ503135	B.A.	N.D.	N.D.	N.D.	Etanol absoluto
(*)	Manantial Prado de Cifuentes, Calatayud, Zaragoza	11/04/2011	30S 612093/4576672	R.M. A.	N.D.	15	1,88	Etanol 70% y absoluto
gasulli	Manantial Rambla de los Yesos, Alboloduy, Almería	15/01/1994 10/08/1996	N.D.	N.M., D.M.	N.D.	N.D.	N.D.	Etanol 70%
(*)	Caño en Cala de San Pedro, Cabo de Gata, Almería	02/05/1997 04/12/2008	N.D.	B.A. J.M.B, D.M.	N.D.	N.D.	N.D.	Etanol 70% y 96%
(*)	Rambla de Retamar, Almería	06/06/2006 08/10/2006	N.D.	D.M., N.M.	N.D.	N.D.	N.D.	Etanol 70%
(*)	Acequia grande en Barranco de las Negras, Cabo de Gata, Almería	29/03/2011	N.D.	D.M.	N.D.	N.D.	N.D.	Etanol 70% y absoluto
	Acequia pequeña y arroyo en Barranco de las Negras, Cabo de Gata, Almería	29/03/2011	N.D.	D.M.	N.D.	N.D.	N.D.	Etanol 70% y absoluto

Apéndice III: Medidas realizadas sobre conchas, opérculos, rádulas y otros órganos internos.

Tabla 1. Medidas realizadas (en mm) sobre las conchas de las especies de *Pseudamnicola*: 1, *P. (C.) luisi* de fuente de La Gitana, La Peza, Granada; 2, *P. (C.) falkneri* de fuente de la Armada, Orce, Granada; 3, *P. (C.) manueli* del arroyo de La Garganta, Nava de San Pedro, Jaén; 4, *P. (C.) bareai* de la fuente de la Ermita de las Santas, Collados de la Sagra, Granada; 5, *P. (C.) marisolae* de la fuente de Pilar del Mono, Dúrcal, Granada; 6, *P. (C.) iruritai* De la fuente de Don Pedro, Loja, Granada; 7, *P. (C.) andalusica* de la fuente de La Salud, Albanchez de Mágina, Jaén; 8, Holotipo y paratipo de *P. (C.) hydrobiopsis* de fuente de la Carmonilla, Loja, Granada.

	1 Mean ± SD; CV (Max-Min) (n=20)	2 Mean ± SD; CV (Max-Min) (n=24)	3 Mean ± SD; CV (Max-Min) (n=25)	4 Mean ± SD; CV (Max-Min) (n=31)	5 Mean ± SD; CV (Max-Min) (n=29)	6 Mean ± SD; CV (Max-Min) (n=17)	7 Mean ± SD; CV (Max-Min) (n=13)	8 Mean ± SD; CV (Max-Min) (n=2)
SL	4,31± 0,46; 0,11 (5,31-3,42)	2,26± 0,17; 0,08 (2,63-2,06)	3,34± 0,23; 0,07 (3,69-2,93)	2,75± 0,22; 0,08 (3,31-2,47)	3,72± 0,29; 0,08 (4,63-3,24)	3,25± 0,31; 0,09 (3,65-2,75)	3,74± 0,32; 0,09 (4,14-3,30)	3,39± 0,33; 0,10 (3,62-3,15)
SW	2,44± 0,18;0,07 (2,70-2,17)	1,24± 0,07; 0,06 (1,42-1,09)	2,11± 0,13; 0,06 (2,27-1,85)	1,88± 0,14; 0,07 (2,17-1,69)	2,06± 0,14; 0,07 (2,48-1,79)	1,71± 0,15; 0,09 (1,99-1,49)	2,20± 0,18; 0,08 (2,42-1,89)	1,46± 0,12; 0,08 (1,54-1,37)
SL/SW	1,77± 0,12; 0,07 (1,97-1,53)	1,76± 0,12; 0,07 (1,94-1,6)	1,58± 0,07; 0,04 (1,78-1,46)	1,47± 0,07; 0,05 (1,60-1,34)	1,80± 0,06; 0,03 (1,90-1,66)	1,89± 0,09; 0,05 (2,01-1,72)	1,70± 0,06; 0,04 (1,79-1,55)	2,32± 0,04; 0,02 (2,35-2,30)
AH	1,97± 0,15; 0,08 (2,33-1,74)	1,01± 0,06; 0,06 (1,15-0,88)	1,63± 0,10; 0,06 (1,77-1,38)	1,42± 0,11; 0,08 (1,64-1,23)	1,66± 0,11; 0,07 (2,01-1,43)	1,37± 0,20; 0,15 (1,95-1,10)	1,72± 0,15; 0,09 (1,92-1,75)	1,06± 0,08; 0,08 (1,12-1,00)
SL-LBW	1,63± 0,32; 0,19 (2,26-1,03)	0,82± 0,11; 0,13 (1,11-0,65)	0,86± 0,13; 0,15 (1,03-0,67)	0,60± 0,11; 0,19 (0,83-0,39)	1,30± 0,15; 0,12 (1,72-1,09)	1,38± 0,18; 0,13 (1,72-1,12)	1,30± 0,20; 0,15 (1,65-1,01)	1,76± 0,35; 0,20 (2,01-1,51)
WBW	2,13± 0,16; 0,08 (2,51-1,87)	1,16± 0,07; 0,06 (1,36-1,05)	1,82± 0,10; 0,05 (1,98-1,60)	1,60± 0,16; 0,10 (1,89-1,00)	1,84± 0,11; 0,06 (2,14-1,61)	1,55± 0,12; 0,08 (1,80-1,36)	1,96± 0,14; 0,07 (2,20-1,74)	1,36± 0,10; 0,07 (1,43-1,29)
AL	1,93± 0,15; 0,08 (2,27-1,67)	1,03± 0,07; 0,06 (1,14-0,87)	1,56± 0,12; 0,08 (1,69-1,19)	1,41± 0,11; 0,07 (1,59-1,22)	1,68± 0,12; 0,07 (1,95-1,44)	1,33± 0,12; 0,09 (1,56-1,18)	1,77± 0,14; 0,08 (1,97-1,56)	1,11± 0,04; 0,04 (1,13-1,07)
AW	1,36± 0,13; 0,09 (1,57-1,13)	0,61± 0,04; 0,07 (0,67-0,51)	1,19± 0,09; 0,08 (1,53-1,04)	1,03± 0,09; 0,09 (1,29-0,87)	1,11± 0,09; 0,08 (1,36-0,96)	0,88± 0,10; 0,11 (1,06-0,71)	1,17± 0,12; 0,10 (1,33-0,98)	0,69± 0,08; 0,12 (0,74-0,63)
WPW	1,55± 0,12; 0,08 (1,75-1,31)	0,91± 0,06; 0,07 (1,04-0,81)	1,21± 0,07; 0,06 (1,35-1,02)	1,04± 0,09; 0,08 (1,26-0,90)	1,32± 0,08; 0,06 (1,57-1,18)	1,19± 0,14; 0,12 (1,47-0,98)	1,43± 0,12; 0,08 (1,66-1,27)	1,25± 0,10; 0,08 (1,33-1,18)
WAW	0,34± 0,10; 0,30 (0,59-0,16)	0,21± 0,05; 0,22 (0,32-0,15)	0,21± 0,08; 0,38 (0,38-0,12)	0,23± 0,03; 0,14 (0,28-0,17)	0,23± 0,04; 0,16 (0,32-0,15)	0,24± 0,06; 0,24 (0,42-0,18)	0,28± 0,09; 0,32 (0,52-0,16)	0,35± 0,01; 0,03 (0,35-0,34)
NSW	4,66± 0,40; 0,09 (5,50-4,00)	4,64± 0,20; 0,04 (5,00-4,50)	4,51± 0,14; 0,03 (4,75-4,25)	4,19± 0,19; 0,05 (4,50-4,00)	4,89± 0,25; 0,05 (5,50-4,50)	4,09± 0,45; 0,11 (5,00-3,00)	4,77± 0,41; 0,09 (5,00-4,00)	5,00± 0,70; 0,14 (5,50-4,50)

Continuación Tabla 1: 9, *P. (C.) astieri* de Source d'Argens, Brue-Aurillac, Var, France; 10, *P. (C.) hauffei* de fuente de los Nogales, Castellón; 11, Fonnueva, Bulbuente, Zaragoza; 12, Pozo Azul en Covanera, Burgos; 13, Fuete María, Ontígola, Toledo; 14, *P. (C.) hinzi* de la balsa de Vargas, Borja, Zaragoza; 15, *P. (C.) hinzi* del parque fluvial de Calamocha, Teruel; 16, *P. (C.) ballestae* n. sp. de fuente en Jatar, Granada.

	9 Mean ± SD; CV (Max-Min) (n=11)	**10** Mean ± SD; CV (Max-Min) (n=18)	**11** Mean ± SD; CV (Max-Min) (n=14)	**12** Mean ± SD; CV (Max-Min) (n=15)	**13** Mean ± SD; CV (Max-Min) (n=25)	**14** Mean ± SD; CV (Max-Min) (n=18)	**15** Mean ± SD; CV (Max-Min) (n=11)	**16** Mean ± SD; CV (Max-Min) (n=13)
SL	3,11± 0,25; 0,08 (3,56-2,58)	2,50± 0,18; 0,07 (2,85-2,17)	3,13± 0,24; 0,08 (3,57-2,91)	2,95± 0,22; 0,08 (3,23-2,50)	4,16± 0,30; 0,07 (4,69-3,64)	3,07± 0,24; 0,08 (3,53-2,75)	3,74± 0,38; 0,10 (4,34-3,13)	4,42± 0,71; 0,16 (5,55-3,26)
SW	1,92± 0,15;0,08 (2,28-1,74)	1,59± 0,10; 0,06 (1,78-1,44)	2,01± 0,09; 0,04 (2,16-1,90)	1,96± 0,13; 0,07 (2,18-1,69)	2,49± 0,15; 0,06 (2,76-2,08)	1,96± 0,13; 0,07 (2,15-1,70)	2,13± 0,17; 0,08 (2,45-1,87)	2,67± 0,31; 0,12 (3,19-2,28)
SL/SW	1,62± 0,10; 0,06 (1,76-1,45)	1,57± 0,07; 0,04 (1,73-1,46)	1,55± 0,08; 0,05 (1,74-1,44)	1,50± 0,07; 0,05 (1,62-1,40)	1,67± 0,08; 0,05 (1,86-1,56)	1,57± 0,09; 0,05 (1,72-1,40)	1,76± 0,08; 0,05 (1,86-1,70)	1,65± 0,11; 0,07 (1,88-1,43)
AH	1,37± 0,14; 0,10 (1,78-1,26)	1,20± 0,08; 0,07 (1,36-1,04)	1,58± 0,09; 0,06 (1,80-1,46)	1,43± 010; 0,07 (1,60-1,20)	2,00± 0,10; 0,05 (2,26-1,81)	1,37± 0,09; 0,07 (1,51-1,17)	1,65± 0,12; 0,07 (1,81-1,42)	2,06± 0,23; 0,11 (2,45-1,69)
SL-LBW	1,10± 0,16; 0,15 (1,39-0,76)	0,69± 0,13; 0,19 (1,00-0,49)	0,89± 0,16; 0,18 (1,32-0,72)	0,80± 0,12; 0,16 (1,06-0,60)	1,31± 0,21; 0,16 (1,71-1,05)	1,07± 0,15; 0,14 (1,32-0,82)	1,31± 0,22; 0,17 (1,62-0,95)	1,59± 0,35; 0,22 (2,08-1,03)
WBW	1,76± 0,09; 0,05 (1,89-1,59)	1,42± 0,07; 0,05 (1,57-1,29)	1,71± 0,08; 0,04 (1,82-1,61)	1,72± 0,11; 0,06 (1,92-1,52)	2,14± 0,14; 0,07 (2,37-1,85)	1,78± 0,17; 0,10 (1,98-1,43)	1,95± 0,15; 0,08 (2,19-1,78)	2,26± 0,27; 0,12 (2,72-1,93)
AL	1,39± 0,12; 0,08 (1,72-1,28)	1,26± 0,09; 0,07 (1,41-1,11)	1,60± 0,09; 0,05 (1,79-1,49)	1,46± 0,10; 0,07 (1,62-1,26)	2,00± 0,12; 0,06 (2,18-1,79)	1,39± 0,09; 0,07 (1,52-1,23)	1,72± 0,12; 0,07 (1,87-1,54)	2,06± 0,24; 0,12 (2,51-1,69)
AW	1,01± 0,14; 0,14 (1,33-0,85)	0,89± 0,08; 0,09 (1,11-0,78)	1,13± 0,07; 0,06 (1,25-1,02)	1,11± 0,11; 0,10 (1,26-0,95)	1,38± 0,12; 0,09 (1,62-1,16)	1,01± 0,08; 0,08 (1,12-0,84)	1,13± 0,09; 0,08 (1,25-0,93)	1,41± 0,18; 0,13 (1,73-0,19)
WPW	1,23± 0,08; 0,07 (1,33-1,11)	0,95± 0,08; 0,08 (1,12-0,80)	1,11± 0,09; 0,08 (1,31-1,03)	1,11± 0,08; 0,07 (1,24-0,96)	1,50± 0,12; 0,08 (1,73-1,28)	1,29± 0,13; 0,10 (1,45-1,05)	1,45± 0,13; 0,09 (1,65-1,32)	1,61± 0,24; 0,15 (1,97-1,28)
WAW	0,19± 0,06; 0,32 (0,32-0,15)	0,28± 0,04; 0,14 (0,37-0,19)	0,18± 0,03; 0,18 (0,25-0,12)	0,23± 0,05; 0,23 (0,36-0,18)	0,36± 0,08; 0,22 (0,50-0,18)	0,19± 0,03; 0,18 (0,25-0,13)	0,33± 0,08; 0,04 (0,48-0,22)	0,34± 0,09; 0,27 (0,48-0,15)
NSW	4,30± 0,31; 0,07 (4,75-4,00)	4,18± 0,21; 0,05 (4,50-4,00)	4,00± 0,00; 0,00 (4,00-4,00)	3,78± 0,25; 0,07 (4,00-3,50)	4,67± 0,15; 0,03 (5,00-4,50)	4,00± 0,00; 0,00 (4,00-4,00)	4,45± 0,15; 0,03 (4,50-4,00)	4,81± 0,35; 0,07 (5,50-4,25)

Continuación Tabla 1. 17, *P. (P.) subproducta* de Ullal de Baltasar, Amposta, Tarragona y 18, Laguna de Ontígola, Madrid; 19, *P. (P.) beckmanni* de Font del Rentador, Deiá, Mallorca; 20, *P. (P.) granjaensis* de la Granja de Esporles, Mallorca; 21, *P. (P.) artanensis* de la acequia de la ermita de Betlem, Artá, Mallorca; 22, *P. (P.) meloussensis* de la cala de la Macarella, Menorca; 23, *P. (P.) gasulli* del Barranco de las Negras, Almería y 24, Rambla de Retamar, Almería.

	17 Mean ± SD; CV (Max-Min) (n=10)	**18** Mean ± SD; CV (Max-Min) (n=20)	**19** Mean ± SD; CV (Max-Min) (n=21)	**20** Mean ± SD; CV (Max-Min) (n=10)	**21** Mean ± SD; CV (Max-Min) (n=25)	**22** Mean ± SD; CV (Max-Min) (n=20)	**23** Mean ± SD; CV (Max-Min) (n=30)	**24** Mean ± SD; CV (Max-Min) (n=30)
SL	2,92± 0,64; 0,22 (3,76-2,12)	2,21± 0,26; 0,12 (2,72-1,75)	2,32± 0,24; 0,10 (2,89-1,95)	4,28± 0,51; 0,12 (5,18-3,64)	3,17± 0,15; 0,05 (3,44-2,87)	2,91± 0,32; 0,11 (3,42-2,30)	2,71± 0,55; 0,20 (3,53-1,86)	3,27± 0,39; 0,12 (4,01-2,42)
SW	2,18± 0,41;0,19 (2,78-1,68)	1,73± 0,17; 0,10 (1,99-1,51)	1,86± 0,16; 0,09 (2,39-1,56)	2,70± 0,26; 0,10 (3,14-2,3)	2,00± 0,07; 0,03 (2,17-1,85)	2,18± 0,19; 0,09 (2,58-1,77)	1,47± 0,20; 0,14 (1,76-1,10)	1,82± 0,18; 0,10 (2,29-1,41)
SL/SW	1,33± 0,08; 0,06 (1,41-1,20)	1,27± 0,07; 0,05 (1,40-1,16)	1,25± 0,07; 0,05 (1,40-1,16)	1,58± 0,09; 0,06 (1,75-1,44)	1,59± 0,07; 0,04 (1,69-1,47)	1,37± 0,05; 0,04 (1,46-1,21)	1,84± 0,23; 0,13 (2,06-1,19)	1,79± 0,08; 0,05 (1,94-1,66)
AH	1,63± 0,30; 0,18 (2,08-1,27)	1,25± 0,14; 0,11 (1,47-1,02)	1,35± 0,13; 0,10 (1,75-1,14)	1,79± 0,18; 0,10 (2,05-1,49)	1,57± 0,09; 0,05 (1,72-1,40)	1,55± 0,15; 0,10 (1,82-1,18)	1,14± 0,20; 0,18 (1,46-0,80)	1,43± 0,13; 0,09 (1,93-1,16)
SL-LBW	0,57± 0,19; 0,32 (0,84-0,31)	0,40± 0,11; 0,27 (0,59-0,24)	0,39± 0,11; 0,27 (0,58-0,27)	1,57± 0,33; 0,21 (2,13-1,13)	0,89± 0,10; 0,11 (1,12-0,70)	0,60± 0,14; 0,24 (0,90-0,36)	1,04± 0,27; 0,26 (1,52-0,76)	1,13± 0,21; 0,19 (1,58-0,69)
WBW	1,81± 0,34; 0,19 (2,35-1,9)	1,48± 0,13; 0,09 (1,71-1,22)	1,50± 0,10; 0,07 (1,72-1,29)	2,37± 0,22; 0,09 (2,69-2,08)	1,73± 0,09; 0,05 (1,98-1,62)	1,82± 0,15; 0,08 (2,12-1,56)	1,34± 0,19; 0,14 (1,58-1,03)	1,71± 0,16; 0,09 (2,01-1,30)
AL	1,62± 0,31; 0,19 (2,05-1,26)	0,90± 0,11; 0,12 (1,09-0,73)	1,35± 0,14; 0,10 (1,74-1,08)	1,74± 0,19; 0,11 (2,06-1,47)	1,58± 0,07; 0,05 (1,68-1,36)	1,54± 0,15; 0,10 (1,90-1,23)	0,75± 0,11; 0,14 (0,95-0,61)	0,94± 0,11; 0,12 (1,29-0,70)
AW	1,19± 0,25; 0,21 (1,54-0,87)	1,20± 0,12; 0,10 (1,42-0,99)	0,99± 0,11; 0,11 (1,26-0,84)	1,43± 0,16; 0,11 (1,69-1,27)	1,14± 0,04; 0,04 (1,21-1,02)	1,17± 0,14; 0,12 (1,49-0,87)	1,22± 0,16; 0,13 (1,44-0,90)	1,43± 0,13; 0,07 (1,83-1,17)
WPW	1,08± 0,26; 0,24 (1,42-0,73)	0,83± 0,12; 0,15 (1,11-0,59)	0,87± 0,08; 0,10 (1,01-0,71)	1,67± 0,16; 0,12 (1,98-1,31)	1,22± 0,06; 0,05 (1,39-1,13)	1,11± 0,14; 0,13 (1,33-0,94)	1,02± 0,14; 0,14 (1,24-0,82)	1,26± 0,13; 0,10 (1,61-0,95)
WAW	0,21± 0,06; 0,29 (0,31-0,10)	0,18± 0,04; 0,24 (0,27-0,11)	0,18± 0,06; 0,32 (0,30-0,08)	0,25± 0,03; 0,12 (0,29-0,20)	0,25± 0,07; 0,28 (0,36-0,15)	0,23± 0,06; 0,26 (0,34-0,12)	0,18± 0,03; 0,19 (0,25-0,14)	0,22± 0,04; 0,20 (0,33-0,16)
NSW	4,21± 0,17; 0,04 (4,50-4,00)	3,74± 0,29; 0,08 (4,25-3,25)	3,99± 0,28; 0,07 (4,25-3,50)	5,08± 0,24; 0,05 (5,50-4,75)	4,24± 0,22; 0,05 (4,50-4,00)	4,21± 0,31; 0,07 (4,50-3,75)	4,92± 1,67; 0,34 (5,50-4,00)	4,58± 0,32; 0,07 (5,00-4,25)

Tabla 2. Medidas (en mm) de los opérculos de las especies de *Pseudamnicola*: 1, *P. (C.) luisi* de fuente de La Gitana, La Peza, Granada; 2, *P. (C.) falkneri* de fuente de la Armada, Orce, Granada; 3, *P. (C.) manueli* del arroyo de La Garganta, Nava de San Pedro, Jaén; 4, *P. (C.) bareai* de la fuente de la Ermita de las Santas, Collados de la Sagra, Granada; 5, *P. (C.) marisolae* De la fuente de Pilar del Mono, Dúrcal, Granada; 6, *P. (C.) iruritai* de la fuente de Don Pedro, Loja, Granada; 7, *P. (C.) andalusica* de la fuente de La Salud, Albanchez de Mágina, Jaén.

	1 Mean ± SD; CV (Max-Min) (n=10)	**2** Mean ± SD; CV (Max-Min) (n=10)	**3** Mean ± SD; CV (Max-Min) (n=8)	**4** Mean ± SD; CV (Max-Min) (n=11)	**5** Mean ± SD; CV (Max-Min) (n=10)	**6** Mean ± SD; CV (Max-Min) (n=4)	**7** Mean ± SD; CV (Max-Min) (n=5)
OL	1,45± 0,10; 0,07 (1,63-1,34)	0,85± 0,06; 0,07 (0,94-0,77)	1,08± 0,12; 0,11 (1,26-0,95)	1,01± 0,08; 0,08 (1,09-0,83)	1,26± 0,11; 0,09 (1,48-1,15)	1,00± 0,06; 0,06 (1,06-0,92)	1,17± 0,11; 0,08 (1,35-1,09)
OW	0,98± 0,06; 0,06 (1,07-0,91)	0,60± 0,05; 0,09 (0,71-0,53)	0,78± 0,06; 0,08 (0,87-0,70)	0,73± 0,06; 0,08 (0,81-0,60)	0,84± 0,06; 0,07 (0,96-0,78)	0,68± 0,03; 0,04 (0,71-0,63)	0,78± 0,07; 0,09 (0,89-0,72)
OLWL	0,69± 0,10; 0,14 (0,87-0,54)	0,43± 0,05; 0,11 (0,50-0,34)	0,59± 0,08; 0,14 (0,70-0,48)	0,54± 0,06; 0,12 (0,61-0,40)	0,67± 0,12; 0,18 (0,87-0,54)	0,54± 0,01; 0,02 (0,54-0,53)	0,70± 0,12; 0,17 (0,86-0,65)
OLWW	0,42± 0,08; 0,18 (0,52-0,31)	0,25± 0,03; 0,14 (0,32-0,21)	0,31± 0,06; 0,18 (0,37-0,23)	0,28± 0,03; 0,11 (0,33-0,23)	0,35± 0,06; 0,17 (0,44-0,29)	0,28± 0,03; 0,11 (0,31-0,25)	0,39± 0,04; 0,10 (0,45-0,36)
NL	0,58± 0,11; 0,20 (0,71-0,41)	0,30± 0,03; 0,09 (0,35-0,27)	0,39± 0,06; 0,16 (0,47-0,30)	0,37± 0,05; 0,13 (0,46-0,29)	0,46± 0,09; 0,20 (0,57-0,33)	0,34± 0,06; 0,18 (0,37-0,25)	0,36± 0,05; 0,14 (0,43-0,30)
NW	0,48± 0,06; 0,12 (0,57-0,40)	0,29± 0,04; 0,13 (0,33-0,23)	0,38± 0,08; 0,22 (0,49-0,22)	0,36± 0,06; 0,16 (0,43-0,23)	0,38± 0,04; 0,11 (0,43-0,30)	0,33± 0,04; 0,12 (0,34-0,28)	0,30± 0,02; 0,07 (0,33-0,27)
OL/OW	1,48± 0,05; 0,03 (1,54-1,41)	1,43± 0,08; 0,06 (1,57-1,32)	1,39± 0,06; 0,04 (1,48-1,32)	1,39± 0,05; 0,04 (1,49-1,31)	1,50± 0,05; 0,03 (1,57-1,42)	1,49± 0,02; 0,01 (1,51-1,46)	1,50± 0,04; 0,03 (1,52-1,43)

Continuación Tabla 2: 8, *P. (C.) astieri* de Source d'Argens, Brue-Aurillac, Var, France; 9, *P. (C.) hauffei* de fuente de los Nogales, Castellón; 10, Fonnueva, Bulbuente, Zaragoza; 11, Pozo Azul en Covanera, Burgos; 12, Fuete María, Ontígola, Toledo; 13, *P. (C.) hinzi* de la balsa de Vargas, Borja, Zaragoza; 14, *P. (C.) hinzi* del parque fluvial de Calamocha, Teruel; 15, *P. (C.) ballestae* n. sp. de fuente en Jatar, Granada.

	8 Mean ± SD; CV (Max-Min) (n=5)	**9** Mean ± SD; CV (Max-Min) (n=7)	**10** Mean ± SD; CV (Max-Min) (n=8)	**11** Mean ± SD; CV (Max-Min) (n=8)	**12** Mean ± SD; CV (Max-Min) (n=10)	**13** Mean ± SD; CV (Max-Min) (n=8)	**14** Mean ± SD; CV (Max-Min) (n=8)	**15** Mean ± SD; CV (Max-Min) (n=7)
OL	1,10± 0,05; 0,05 (1,18-0,96)	1,08± 0,08; 0,08 (1,18-0,96)	1,16± 0,26; 0,22 (1,54-0,89)	1,18± 0,09; 0,08 (1,28-1,03)	1,30± 0,10; 0,07 (1,45-1,17)	1,00± 0,11; 0,11 (1,15-0,88)	1,12± 0,09; 0,08 (1,31-1,04)	1,28± 0,06; 0,04 (1,41-1,25)
OW	0,79± 0,04;0,05 (0,84-0,73)	0,74± 0,06; 0,08 (0,84-0,66)	0,81± 0,16; 0,20 (1,04-0,62)	0,83± 0,05; 0,07 (0,89-0,75)	0,94± 0,06; 0,06 (1,01-0,87)	0,72± 0,07; 0,09 (0,81-0,64)	0,80± 0,07; 0,09 (0,94-0,70)	0,92± 0,10; 0,11 (1,18-0,82)
OLWL	0,48± 0,05; 0,11 (0,56-0,40)	0,49± 0,08; 0,17 (0,63-0,36)	0,53± 0,14; 0,27 (0,84-0,40)	0,59± 0,11; 0,18 (0,71-0,45)	0,63± 0,13; 0,20 (0,88-0,47)	0,51± 0,09; 0,17 (0,64-0,40)	0,61± 0,05; 0,08 (0,67-0,54)	0,78± 0,04; 0,05 (0,86-0,74)
OLWW	0,34± 0,05; 0,16 (0,40-0,25)	0,33± 0,03; 0,09 (0,38-0,29)	0,36± 0,09; 0,24 (0,51-0,25)	0,37± 0,05; 0,13 (0,43-0,30)	0,44± 0,06; 0,13 (0,51-0,34)	0,34± 0,04; 0,12 (0,40-0,27)	0,37± 0,05; 0,13 (0,46-0,30)	0,47± 0,02; 0,05 (0,52-0,44)
NL	0,47± 0,04; 0,09 (0,52-0,39)	0,36± 0,06; 0,17 (0,44-0,26)	0,45± 0,14; 0,31 (0,68-0,26)	0,41± 0,05; 0,12 (0,49-0,33)	0,45± 0,04; 0,09 (0,51-0,35)	0,32± 0,05; 0,17 (0,40-0,25)	0,39± 0,05; 0,13 (0,46-0,33)	0,39± 0,10; 0,25 (0,46-0,26)
NW	0,36± 0,04; 0,12 (0,42-0,31)	0,29± 0,06; 0,222 (0,45-0,21)	0,36± 0,08; 0,22 (0,45-0,24)	0,37± 0,04; 0,10 (0,43-0,27)	0,42± 0,03; 0,07 (0,47-0,30)	0,29± 0,04; 0,16 (0,34-0,24)	0,42± 0,07; 0,16 (0,52-0,35)	0,37± 0,05; 0,15 (0,42-0,30)
OL/OW	1,40± 0,05; 0,08 (1,49-1,34)	1,44± 0,06; 0,08 (1,52-1,37)	1,43± 0,07; 0,05 (1,57-1,38)	1,42± 0,04; 0,03 (1,54-1,36)	1,38± 0,05; 0,03 (1,45-1,32)	1,39± 0,07; 0,05 (1,51-1,31)	1,40± 0,07; 0,05 (1,54-1,35)	1,41± 0,12; 0,08 (1,54-1,19)

Continuación Tabla 2: 16, *P. (P.) subproducta* de Ullal de Baltasar, Amposta, Tarragona y 17, Laguna de Ontígola, Madrid; 18, *P. (P.) beckmanni* de Font del Rentador, Deiá, Mallorca; 19, *P. (P.) granjaensis* de la Granja de Esporles, Mallorca; 20, *P. (P.) artanensis* de la acequia de la ermita de Betlem, Artá, Mallorca; 21, *P. (P.) meloussensis* de la cala de la Macarella, Menorca; 22, *P. (P.) gasulli* del Barranco de las Negras y 23 Rambla de Retamar, Almería.

	16 Mean ± SD; CV (Max-Min) (n=3)	**17** Mean ± SD; CV (Max-Min) (n=5)	**18** Mean ± SD; CV (Max-Min) (n=8)	**19** Mean ± SD; CV (Max-Min) (n=5)	**20** Mean ± SD; CV (Max-Min) (n=7)	**21** Mean ± SD; CV (Max-Min) (n=10)	**22** Mean ± SD; CV (Max-Min) (n=7)	**23** Mean ± SD; CV (Max-Min) (n=6)
OL	1,44± 0,05; 0,03 (1,48-1,40)	1,24± 0,13; 0,10 (1,33-1,03)	1,18± 0,08; 0,06 (1,33-1,11)	1,75± 0,29; 0,16 (2.13-1,47)	1,27± 0,08; 0,06 (1,33-1,12)	1,66± 0,15; 0,09 (1,94-1,50)	0,78± 0,10; 0,12 (0,91-0,68)	1,07± 0,05; 0,04 (1,13-1,01)
OW	0,97± 0,04;0,04 (1,00-0,93)	0,90± 0,10; 0,11 (0,98-0,74)	0,87± 0,07; 0,08 (0,98-0,77)	1,22± 0,18; 0,15 (1,49-1,03)	0,84± 0,06; 0,07 (0,93-0,77)	1,21± 0,12; 0,10 (1,39-1,07)	0,55± 0,07; 0,12 (0,62-0,46)	0,69± 0,04; 0,06 (0,76-0,65)
OLWL	0,80± 0,06; 0,08 (0,86-0,75)	0,68± 0,09; 0,13 (0,73-0,54)	0,62± 0,05; 0,08 (0,71-0,53)	0,91± 0,20; 0,21 (1,07-0,65)	0,77± 0,08; 0,11 (0,85-0,66)	0,93± 0,09; 0,10 (1,07-0,78)	0,45± 0,08; 0,17 (0,54-0,33)	0,67± 0,06; 0,08 (0,75-0,61)
OLWW	0,46± 0,01; 0,03 (0,47-0,45)	0,44± 0,08; 0,17 (0,54-0,34)	0,44± 0,08; 0,18 (0,57-0,35)	0,65± 0,11; 0,17 (0,82-0,55)	0,40± 0,05; 0,13 (0,48-0,33)	0,62± 0,10; 0,16 (0,74-0,51)	0,25± 0,09; 0,35 (0,35-0,13)	0,27± 0,09; 0,31 (0,39-0,18)
NL	0,39± 0,04; 0,10 (0,41-0,35)	0,35± 0,08; 0,22 (0,43-0,28)	0,40± 0,05; 0,13 (0,46-0,34)	0,48± 0,06; 0,12 (0,51-0,38)	0,36± 0,03; 0,09 (0,40-0,32)	0,48± 0,07; 0,15 (0,62-0,42)	0,27± 0,04; 0,14 (0,30-0,19)	0,31± 0,04; 0,11 (0,36-0,27)
NW	0,41± 0,03; 0,07 (0,44-0,39)	0,35± 0,07; 0,19 (0,42-0,27)	0,40± 0,04; 0,11 (0,44-0,33)	0,53± 0,10; 0,19 (0,63-0,38)	0,36± 0,05; 0,13 (0,42-0,31)	0,54± 0,05; 0,08 (0,62-0,49)	0,27± 0,03; 0,13 (0,34-0,24)	0,34± 0,06; 0,17 (0,41-0,27)
OL/OW	1,49± 0,11; 0,07 (1,59-1,40)	1,38± 0,07; 0,05 (1,46-1,28)	1,37± 0,04; 0,03 (1,44-1,31)	1,44± 0,23; 0,16 (1,78-1,25)	1,52± 0,09; 0,06 (1,64-1,42)	1,38± 0,05; 0,03 (1,42-1,29)	1,44± 0,11; 0,08 (1,57-1,26)	1,54± 0,11; 0,07 (1,69-1,41)

Tabla 3. Fórmula y medidas de la rádula de las especies de *Pseudamnicola*: 1, *P. (C.) luisi* de fuente de La Gitana, La Peza, Granada; 2, *P. (C.) falkneri* de fuente de la Armada, Orce, Granada; 3, *P. (C.) manueli* del arroyo de La Garganta, Nava de San Pedro, Jaén; 4, *P. (C.) bareai* de la fuente de la Ermita de las Santas, Collados de la Sagra, Granada; 5, *P. (C.) marisolae* de la fuente de Pilar del Mono, Dúrcal, Granada; 6, *P. (C.) iruritai* de la fuente de Don Pedro, Loja, Granada; 7, *P. (C.) andalusica* de la fuente de La Salud, Albanchez de Mágina, Jaén.

	1	2	3	4	5	6	7
Fórmula diente central	3+C+3/1-1	0/1-1	(5)-4+C+(5)-4/1-1	(4)-5+C+5-(6)/1-1	(3)-4+C+4-(3)/1-1	6+C+6/1-1	4+C+4/1-1
Ancho diente central	~ 35 µm	~ 15 µm	~ 20 µm	~ 20 µm	~ 30 µm	~ 20 µm	~ 25 µm
Fórmula diente lateral	2-C-2	6-C-6	3-C-3	4-C-4	2-C-2	3/4-C-3/4	3-C-3
Número de cúspides diente marginal interno	≥ 12	≥ 33	≥ 18	≥ 18	≥ 11	≥ 20	≥ 13
Número de cúspides diente marginal externo	≥ 15	≥ 25	≥ 22	≥ 20	≥ 9	≥ 25	≥ 15
Longitud rádula	~870 µm	~670 µm	~590 µm	~ 710 µm	~ 800 µm	~ 700 µm	~ 850 µm
Ancho rádula	~ 110 µm	~ 80 µm	~ 90 µm	~ 90 µm	~ 100 µm	~ 95 µm	~ 90 µm
Número de filas de dientes	~ 50	~ 60	~ 50	~ 60	~ 50	~ 50	~ 45

Continuación Tabla 3. 8, *P. (C.) astieri* de Source d'Argens, Brue-Aurillac, Var, France; 9, *P. (C.) hauffei* de fuente de los Nogales, Castellón; 10, *P. (C.) navasiana* de Fonnueva, Bulbuente, Zaragoza y 11, Pozo Azul en Covanera, Burgos; 12, *P. (C.) hinzi* de la balsa de Vargas, Borja, Zaragoza; 13, *P. (C.) ballestae* n. sp. de fuente en Jatar, Granada.

	8	9	10	11	12	13
Fórmula diente central	7+C+7/1-1	5+C+5/1-1	5+C+5/1-1	5+C+5/1-1	6+C+6/1-1	(5)-4+C+4-(5)/1-1
Ancho diente central	~ 20 µm	~ 15 µm	~ 30 µm	~ 25 µm	~ 20 µm	~ 40 µm
Fórmula diente lateral	3-C-3	3-C-3	(4)/3-C-3/(4)	(4)/3-C-3/(4)	4-C-4	3-C-3/(2)
Número de cúspides diente marginal interno	≥ 18	≥ 15	≥ 20	≥ 18	≥ 23 c	≥ 15
Número de cúspides diente marginal externo	≥ 19	≥ 19	≥ 22	≥ 20	≥ 28	≥ 20
Longitud rádula	~700 µm	~600 µm	~ 800 µm	~ 700 µm	~ 700 µm	~ 1 mm
Ancho rádula	~ 90 µm	~ 95 µm	~ 100 µm	~ 100 µm	~ 85 µm	~ 120 µm
Número de filas de dientes	~ 55	~ 50	~ 55	~ 50	~ 65	~ 60

Continuación Tabla 3. 14, *P. (P.) subproducta* de Ullal de Baltasar, Amposta, Tarragona; 15, *P. (P.) beckmanni* de Font del Rentador, Deiá, Mallorca; 16, *P. (P.) granjaensis* de la Granja de Esporles, Mallorca; 17, *P. (P.) artanensis* de la acequia de la ermita de Betlem, Artá, Mallorca; 18, *P. (P.) meloussensis* de la cala de la Macarella, Menorca; 19, Barranco de las Negras, Almería.

	14	**15**	**16**	**17**	**18**	**19**
Fórmula diente central	5+C+5/1-1	5+C+5/1-1	5+C+5/1-1	6+C+6/1-1	5+C+5/1-1	4(5)+C+4(5)/1-1
Ancho diente central	~ 25 µm	~ 20 µm	~ 25 µm	~ 25 µm	~ 20 µm	~ 25 µm
Fórmula diente lateral	3-C-3	3-C-3	3-C-3	3-C-3	3-C-3	4-C-4
Número de cúspides diente marginal interno	≥ 20	≥ 15	≥ 15	≥ 20	≥ 18	≥ 20
Número de cúspides diente marginal externo	≥ 19	≥ 20	≥ 15	≥ 20	≥ 12	≥ 22
Longitud rádula	~500 µm	~550 µm	~ 850 µm	~ 400 µm	~ 700 µm	~ 500 µm
Ancho rádula	~ 120 µm	~ 90 µm	~ 150 µm	~ 100 µm	~ 100 µm	~ 100 µm
Número de filas de dientes	~ 35	~ 55	~ 55	~ 35	~ 50	~ 50

Tabla 4. Medidas (en mm) del ctenidio, osfradio y estómago de las especies de *Pseudamnicola*: 1, *P. (C.) luisi* de fuente de La Gitana, La Peza, Granada; 2, *P. (C.) falkneri* de fuente de la Armada, Orce, Granada; 3, *P. (C.) manueli* del arroyo de La Garganta, Nava de San Pedro, Jaén; 4, *P. (C.) bareai* de la fuente de la Ermita de las Santas, Collados de la Sagra, Granada; 5, *P. (C.) marisolae* de la fuente de Pilar del Mono, Dúrcal, Granada; 6, *P. (C.) iruritai* de la fuente de Don Pedro, Loja, Granada; 7, *P. (C.) andalusica* de la fuente de La Salud, Albanchez de Mágina, Jaén.

	1	**2**	**3**	**4**	**5**	**6**	**7**
	Mean ± SD; CV (Max-Min) (n=8)	Mean ± SD; CV (Max-Min) (n=8)	Mean ± SD; CV (Max-Min) (n=12)	Mean ± SD; CV (Max-Min) (n=10)	Mean ± SD; CV (Max-Min) (n=10)	Mean ± SD; CV (Max-Min) (n=7)	Mean ± SD; CV (Max-Min) (n=5)
CL	1,19± 0,18; 0,15 (1,38-0,94)	0,86± 0,10; 0,11 (0,98-0,72)	1,43± 0,15; 0,11 (1,69-1,19)	1,05± 0,19; 0,19 (1,28-0,78)	1,26± 0,21; 0,17 (1,62-1,00)	0,76± 0,11; 0,15 (0,90-0,61)	0,98± 0,10; 0,10 (1,19-0,93)
Os L	0,46± 0,08; 0,17 (0,56-0,34)	0,27± 0,04; 0,16 (0,34-0,21)	0,41± 0,07; 0,17 (0,57-0,34)	0,29± 0,02; 0,07 (0,31-0,25)	0,33± 0,10; 0,31 (0,51-0,20)	0,29± 0,04; 0,13 (0,33-0,22)	0,28± 0,06; 0,22 (0,39-0,21)
Os W	0,23± 0,06; 0,28 (0,35-0,18)	0,10± 0,02; 0,25 (0,14-0,06)	0,20± 0,03; 0,18 (0,24-0,12)	0,15± 0,02; 0,13 (0,17-0,11)	0,10± 0,02; 0,16 (0,13-0,08)	0,14± 0,04; 0,26 (0,20-0,09)	0,11± 0,03; 0,31 (0,17-0,07)
Ss L	0,90± 0,07; 0,08 (1,00-0,79)	0,43± 0,05; 0,11 (0,49-0,37)	0,64± 0,07; 0,11 (0,73-0,50)	0,62± 0,07; 0,12 (0,69-0,44)	0,72± 0,09; 0,12 (0,82-0,57)	0,48± 0,05; 0,10 (0,55-0,41)	0,69± 0,09; 0,13 (0,80-0,61)
Ss W	0,57± 0,10; 0,17 (0,74-0,44)	0,26± 0,03; 0,12 (0,30-0,20)	0,41± 0,04; 0,10 (0,45-0,34)	0,35± 0,05; 0,15 (0,41-0,27)	0,45± 0,08; 0,17 (0,54-0,32)	0,32± 0,04; 0,13 (0,37-0,28)	0,43± 0,03; 0,06 (0,47-0,41)
St L	0,98± 0,08; 0,09 (1,13-0,91)	0,60± 0,04; 0,07 (0,67-0,53)	0,78± 0,06; 0,07 (0,86-0,67)	0,61± 0,08; 0,13 (0,72-0,48)	0,87± 0,10; 0,12 (1,08-0,75)	0,61± 0,06; 0,09 (0,70-0,53)	0,77± 0,05; 0,07 (0,86-0,72)
St W	0,93± 0,10; 0,11 (1,06-0,72)	0,54± 0,07; 0,13 (0,65-0,44)	0,77± 0,07; 0,08 (0,86-0,63)	0,67± 0,06; 0,08 (0,74-0,58)	0,75± 0,10; 0,13 (0,89-0,60)	0,56± 0,03; 0,07 (0,61-0,53)	0,73± 0,05; 0,07 (0,81-0,68)

Continuación Tabla 4. 8, *P. (C.) astieri* de Source d'Argens, Brue-Aurillac, Var, France; 9, *P. (C.) hauffei* de fuente de los Nogales, Castellón; 10, *P. (C.) navasiana* de Fonnueva, Bulbuente, Zaragoza, 11, Pozo Azul en Covanera, Burgos y 12, Fuete María, Ontígola, Toledo; 13, *P. (C.) hinzi* de la balsa de Vargas, Borja, Zaragoza; 14, *P. (C.) hinzi* del parque fluvial de Calamocha, Teruel; 15, *P. (C.) ballestae* n. sp. de fuente en Jatar, Granada.

	8 Mean ± SD; CV (Max-Min) (n=5)	**9** Mean ± SD; CV (Max-Min) (n=7)	**10** Mean ± SD; CV (Max-Min) (n=14)	**11** Mean ± SD; CV (Max-Min) (n=3)	**12** Mean ± SD; CV (Max-Min) (n=5)	**13** Mean ± SD; CV (Max-Min) (n=9)	**14** Mean ± SD; CV (Max-Min) (n=6)	**15** Mean ± SD; CV (Max-Min) (n=8)
CL	1,06± 0,15; 0,13 (1,25-0,90)	1,11± 0,16; 0,14 (1,27-0,92)	0,89± 0,13; 0,15 (1,20-0,71)	0,97± 0,08; 0,08 (1,03-0,89)	1,59± 0,24; 0,15 (1,83-1,26)	1,02± 0,16; 0,16 (1,23-0,80)	0,90± 0,20; 0,22 (1,20-0,72)	1,39± 0,15; 0,11 (1,60-1,25)
Os L	0,32± 0,09; 0,27 (0,43-0,20)	0,33± 0,07; 0,22 (0,45-0,24)	0,32± 0,06; 0,19 (0,40-0,21)	0,29± 0,02; 0,08 (0,31-0,27)	0,51± 0,10; 0,19 (0,61-0,42)	0,32± 0,08; 0,24 (0,40-0,20)	0,28± 0,04; 0,15 (0,34-0,24)	0,40± 0,07; 0,17 (0,51-0,30)
Os W	0,13± 0,02; 0,16 (0,15-0,11)	0,21± 0,03; 0,15 (0,25-0,15)	0,14± 0,04; 0,29 (0,20-0,06)	0,14± 0,04; 0,24 (0,18-0,11)	0,16± 0,03; 0,17 (0,19-0,12)	0,14± 0,04; 0,28 (0,23-0,10)	0,11± 0,03; 0,26 (0,15-0,07)	0,18± 0,05; 0,30 (0,28-0,12)
Ss L	0,69± 0,06;0,11 (0,67-0,50)	0,62± 0,03; 0,05 (0,66-0,58)	0,63± 0,08; 0,12 (0,74-0,53)	0,57± 0,08; 0,14 (0,61-0,49)	0,75± 0,15; 0,20 (1,00-0,66)	0,59± 0,06; 0,10 (0,71-0,54)	0,65± 0,07; 0,11 (0,78-0,60)	0,86± 0,10; 0,12 (0,94-0,62)
Ss W	0,35± 0,02; 0,06 (0,37-0,31)	0,37± 0,03; 0,08 (0,41-0,33)	0,36± 0,05; 0,15 (0,51-0,30)	0,30± 0,06; 0,19 (0,35-0,25)	0,49± 0,05; 0,10 (0,56-0,44)	0,36± 0,04; 0,10 (0,41-0,30)	0,38± 0,04; 0,10 (0,45-0,35)	0,54± 0,07; 0,14 (0,62-0,40)
St L	0,71± 0,04; 0,06 (0,74-0,66)	0,73± 0,06; 0,09 (0,84-0,66)	0,77± 0,22; 0,29 (1,21-0,51)	0,64± 0,04; 0,06 (0,67-0,60)	0,84± 0,13; 0,16 (1,00-0,72)	0,70± 0,04; 0,06 (0,76-0,64)	0,77 ± 0,07; 0,09 (0,88-0,67)	1,03± 0,12; 0,11 (1,19-0,81)
St W	0,61± 0,04; 0,07 (0,67-0,56)	0,69± 0,06; 0,09 (0,78-0,61)	0,64± 0,03; 0,04 (0,72-0,61)	0,58± 0,13; 0,22 (0,66-0,45)	0,62± 0,08; 0,12 (0,69-0,51)	0,58± 0,06; 0,11 (0,66-0,48)	0,69± 0,06; 0,09 (0,78-0,61)	1,00± 0,11; 0,12 (1,11-0,77)

Continuación Tabla 4. 16, *P. (P.) subproducta* de Ullal de Baltasar, Amposta, Tarragona y 17, Laguna de Ontígola, Madrid; 18, *P. (P.) beckmanni* de Font del Rentador, Deiá, Mallorca; 19, *P. (P.) granjaensis* de la Granja de Esporles, Mallorca; 20, *P. (P.) artanensis* de la acequia de la ermita de Betlem, Artá, Mallorca; 21, *P. (P.) meloussensis* de la cala de la Macarella, Menorca; 22, *P. (P.) gasulli* del barranco de las Negras, Almería y 23, Rambla de los yesos, Alboloduy, Almería.

	16 Mean ± SD; CV (Max-Min) (n=6)	**17** Mean ± SD; CV (Max-Min) (n=6)	**18** Mean ± SD; CV (Max-Min) (n=7)	**19** Mean ± SD; CV (Max-Min) (n=3)	**20** Mean ± SD; CV (Max-Min) (n=6)	**21** Mean ± SD; CV (Max-Min) (n=10)	**22** Mean ± SD; CV (Max-Min) (n=8)	**23** Mean ± SD; CV (Max-Min) (n=12)
CL	0,92± 0,16; 0,17 (1,17-0,70)	1,03± 0,04; 0,04 (1,09-1,00)	0,83± 0,07; 0,09 (0.92-0,72)	1.52± 0,41; 0,27 (2,02-1.17)	1,39± 0,26; 0,19 (1,66-1,05)	1,26± 0,15; 0,12 (1,50-1.08)	0,87± 0,12; 0,14 (1,02-0,70)	0,72± 0,09; 0,12 (0,80-0,53)
Os L	0,31± 0,13; 0,41 (0,56-0,16)	0,41± 0,09; 0,22 (0,57-0,33)	0,26± 0,05; 0,18 (0,34-0,19)	0,59± 0,10; 0,17 (0,70-0,48)	0,37± 0,07; 0,20 (0,50-0,30)	0,40± 0,07; 0,19 (0,50-0,26)	0,25± 0,06; 0,23 (0,35-0,20)	0,23± 0,07; 0,30 (0,36-0,16)
Os W	0,11± 0,04; 0,32 (0,18-0,07)	0,13± 0,01; 0,11 (0,15-0,11)	0,09± 0,03; 0,32 (0,12-0,05)	0,23± 0,19; 0,55 (0,26-0,20)	0,14± 0,02; 0,13 (0,17-0,12)	0,15± 0,03; 0,22 (0,19-0,08)	0,10± 0,02; 0,18 (0,13-0,08)	0,10± 0,03; 0,32 (0,15-0,06)
Ss L	0,55± 0,16;0,29 (0,85-0,38)	0,57± 0,10; 0,17 (0,68-0,43)	0,49± 0,06; 0,13 (0,63-0,44)	0,90± 0,06; 0,06 (0,95-0,83)	0,58± 0,03; 0,06 (0.62-0,53)	0,72± 0,07; 0,10 (0,83-0,59)	0,47± 0,06; 0,13 (0,57-0,36)	0,43± 0,10; 0,23 (0,62-0,30)
Ss W	0,38± 0,10; 0,26 (0,56-0,27)	0,45± 0,11; 0,24 (0,63-0,35)	0,34± 0,07; 0,21 (0,51-0,30)	0,65± 0,11; 0,16 (0,73-0,52)	0,43± 0,04; 0,10 (0,50-0,38)	0,43± 0,04; 0,09 (0,49-0,37)	0,31± 0,04; 0,14 (0,36-0,23)	0,29± 0,08; 0,27 (0,41-0,14)
St L	0,72± 0,14; 0,19 (0,94-0,59)	0,70± 0,07; 0,10 (0,80-0,60)	0,57± 0,10; 0,17 (0.67-0,31)	1.21± 0,25; 0,21 (1,44-0,91)	0,77± 0,09; 0,12 (0.87-0,64)	0,80± 0,08; 0,10 (0,87-0,64)	0,59 ± 0,09; 0,16 (0,74-0,50)	0,54± 0,10; 0,18 (0,73-0,43)
St W	0,61± 0,14; 0,22 (0,85-0,48)	0,65± 0,06; 0,09 (0,75-0,60)	0,57± 0,10; 0,17 (0,72-0,48)	1.06± 0,11; 0,11 (1.20-0,96)	0,72± 0,05; 0,07 (0,78-0,66)	0,71± 0,07; 0,09 (0,78-0,60)	0,55± 0,04; 0,07 (0,59-0,48)	0,51± 0,10; 0,20 (0,72-0,41)

Tabla 5. Medidas (en mm) del sistema genital femenino de las especies de *Pseudamnicola*: 1, *P. (C.) luisi* de fuente de La Gitana, La Peza, Granada; 2, *P. (C.) falkneri* de fuente de la Armada, Orce, Granada; 3, *P. (C.) manueli* del arroyo de La Garganta, Nava de San Pedro, Jaén; 4, *P. (C.) bareai* de la fuente de la Ermita de las Santas, Collados de la Sagra, Granada; 5, *P. (C.) marisolae* de la fuente de Pilar del Mono, Dúrcal, Granada; 6, *P. (C.) iruritai* de la fuente de Don Pedro, Loja, Granada; 7, *P. (C.) andalusica* de la fuente de La Salud, Albanchez de Mágina, Jaén.

	1 Mean ± SD; CV (Max-Min) (n=4)	**2** Mean ± SD; CV (Max-Min) (n=4)	**3** Mean ± SD; CV (Max-Min) (n=5)	**4** Mean ± SD; CV (Max-Min) (n=5)	**5** Mean ± SD; CV (Max-Min) (n=5)	**6** Mean ± SD; CV (Max-Min) (n=4)	**7** Mean ± SD; CV (Max-Min) (n=3)
Po L	2,33± 0,37; 0,15 (2,80-2,03)	1,66± 0,26; 0,16 (1,89-1,35)	2,25± 0,29; 0,13 (2,53-1,89)	1,95± 0,17; 0,09 (2,17-1,80)	2,84± 0,20; 0,09 (3,00-2,60)	1,74± 0,28; 0,16 (1,98-1,49)	1,79± 0,56; 0,31 (2,30-1,30)
Po W	0,59± 0,18; 0,30 (0,81-0,44)	0,36± 0,04; 0,10 (0,41-0,34)	0,60± 0,01; 0,01 (0,61-0,59)	0,50± 0,04; 0,08 (0,56-0,45)	0,70± 0,12; 0,18 (0,85-0,55)	0,49± 0,08;0,16 (0,60-0,40)	0,66± 0,19; 0,30 (0,80-0,50)
Ag, L	1,14± 0,26; 0,23 (1,50-1,00)	0,76± 0,20; 0,26 (0,92-0,50)	0,99± 0,18; 0,18 (1,19-0,76)	0,89± 0,06; 0,07 (0,94-0,80)	1,34± 0,05; 0,04 (1,39-1,27)	0,73± 0,11; 0,15 (0,88-0,65)	0,63± 0,22; 0,36 (0,77-0,40)
Cg, L	1,19± 0,13; 0,11 (1,31-1,03)	0,90± 0,08; 0,09 (0,97-0,83)	1,22± 0,11; 0,09 (1,34-1,10)	1,01± 0,09; 0,09 (1,12-0,89)	1,48± 0,18; 0,13 (1,62-1,20)	0,87± 0,19; 0,22 (1,10-0,69)	1,23± 0,51; 0,41 (1,50-0,71)
SR1 L	0,33± 0,05; 0,17 (0,38-0,26)	0,14± 0,05; 0,35 (0,21-0,11)	0,18± 0,05; 0,27 (0,24-0,14)	0,13± 0,05; 0,37 (0,20-0,09)	0,21± 0,04; 0,17 (0,25-0,16)	0,24± 0,02; 0,06 (0,25-0,22)	0,21± 0,03; 0,16 (0,24-0,18)
BC L	1,49± 0,41; 0,28 (1,95-1,04)	1,14± 0,16; 0,15 (1,30-0,95)	1,11± 0,15; 0,13 (1,90-0,95)	1,33± 0,28; 0,21 (1,60-0,97)	1,05± 0,10; 0,09 (1,21-0,98)	0,59± 0,16; 0,27 (0,80-0,47)	0,71± 0,12; 0,16 (0,80-0,60)
BC W	0,42± 0,08; 0,20 (0,49-0,33)	0,26± 0,07; 0,28 (0,34-0,20)	0,37± 0,07; 0,18 (0,47-0,37)	0,35± 0,09; 0,26 (0,45-0,26)	0,36± 0,06; 0,16 (0,45-0,32)	0,33± 0,05; 0,14 (0,39-0,29)	0,26± 0,03; 0,10 (0,29-0,25)
dBC L	0.68± 0.18; 0.26 (0.90-0.55)	0.70± 0.06; 0.09 (0.78-0.66)	0.53± 0.04; 0.08 (0.58-0.47)	0.53± 0.05; 0.10 (0.60-0.48)	0.84± 0.17; 0.20 (1.06-0.62)	0.33± 0.07; 0.20 (0.40-0.26)	0.61± 0.15; 0.25 (0.69-0.45)

Continuación Tabla 5. 8, *P. (C.) astieri* de Source d'Argens, Brue-Aurillac, Var, France; 9, *P. (C.) hauffei* de fuente de los Nogales, Castellón; 10, *P. (C.) navasiana* de Fonnueva, Bulbuente, Zaragoza, 11, Pozo Azul en Covanera, Burgos y 12, Fuete María, Ontígola, Toledo; 13, *P. (C.) hinzi* de la balsa de Vargas, Borja, Zaragoza; 14, *P. (C.) hinzi* del parque fluvial de Calamocha, Teruel; 15, *P. (C.) ballestae* n. sp. de fuente en Jatar, Granada.

	8 Mean ± SD; CV (Max-Min) (n=2)	**9** Mean ± SD; CV (Max-Min) (n=4)	**10** Mean ± SD; CV (Max-Min) (n=9)	**11** Mean ± SD; CV (Max-Min) (n=2)	**12** Mean ± SD; CV (Max-Min) (n=2)	**13** Mean ± SD; CV (Max-Min) (n=3)	**14** Mean ± SD; CV (Max-Min) (n=3)	**15** Mean ± SD; CV (Max-Min) (n=4)
Po L	2,22± 0,24; 0,11 (2,35-2,08)	2,04± 0,26; 0,13 (2,32-1,75)	1,74± 0,37; 0,21 (2,50-1,30)	1,95± 0,24; 0,12 (2,08-1,81)	1,74± 0,25; 0,14 (1,88-1,60)	1,89± 0,12; 0,07 (2,01-1,80)	1,82± 0,18; 0,10 (2,00-1,70)	2,79± 0,03; 0,01 (2,83-2,76)
Po W	0,57± 0,03; 0,04 (0,59-0,56)	0,45± 0,04; 0,10 (0,49-0,41)	0,50± 0,11; 0,22 (0,66-0,38)	0,64± 0,06; 0,10 (0,60-0,67)	0,53± 0,18; 0,33 (0,63-0,43)	0,48± 0,01; 0,02 (0,49-0,47)	0,66± 0,07; 0,10 (0,72-0,60)	0,81± 0,10; 0,12 (0,90-0,70)
Ag, L	0,95± 0,18; 0,18 (1,04-0,84)	0,81± 0,18; 0,24 (1,00-0,71)	0,65± 0,20; 0,31 (1,00-0,44)	0,75± 0,16; 0,21 (0,84-0,66)	0,67± 0,06; 0,09 (0,70-0,63)	0,65± 0,04; 0,06 (0,68-0,61)	0,69± 0,11; 0,16 (0,80-0,62)	1,22± 0,07; 0,06 (1,29-1,14)
Cg, L	1,01± 0,18; 0,17 (1,11-0,91)	1,00± 0,09; 0,09 (1,11-0,93)	0,78± 0,18; 0,23 (1,10-0,58)	1,01± 0,19; 0,19 (1,12-0,90)	0,92± 0,21; 0,23 (1,04-0,80)	0,97± 0,12; 0,12 (1,07-0,86)	0,86± 0,08; 0,09 (0,90-0,78)	1,48± 0,08; 0,06 (1,59-1,43)
SR1 L	0,14± 0,00; 0,00 (0,15-0,14)	0,18± 0,05; 0,18 (0,22-0,15)	0,15± 0,03; 0,20 (0,20-0,11)	0,15± 0,03; 0,18 (0,16-0,13)	0,11± 0,02; 0,16 (0,12-0,10)	0,16± 0,02; 0,11 (0,18-0,15)	0,17± 0,02; 0,10 (0,18-0,15)	0,32± 0,04; 0,14 (0,35-0,26)
BC L	1,24± 0,15; 0,12 (1,32-1,15)	1,37± 0,30; 0,22 (1,56-0,95)	0,98± 0,15; 0,16 (1,19-0,72)	0,89± 0,24; 0,27 (1,02-0,75)	0,97± 0,12; 0,13 (1,04-0,90)	1,19± 0,07; 0,06 (1,26-1,13)	1,04± 0,06; 0,06 (1,10-1,01)	1,58± 0,26; 0,16 (1,89-1,35)
BC W	0,30± 0,09; 0,29 (0,35-0,22)	0,31± 0,05; 0,18 (0,36-0,25)	0,24± 0,07; 0,27 (0,93-0,50)	0,20± 0,02; 0,09 (0,21-0,19)	0,26± 0,06; 0,24 (0,29-0,22)	0,27± 0,09; 0,34 (0,36-0,20)	0,27± 0,10; 0,38 (0,35-0,17)	0,50± 0,10; 0,20 (0,60-0,39)
dBC L	0,60± 0,09; 0,15 (0,75-0,65)	0,62± 0,13; 0,21 (0,72-0,46)	0,69± 0,17; 0,24 (0,93-0,50)	0,51± 0,19; 0,38 (0,62-0,40)	0,50± 0,14; 0,28 (0,58-0,42)	0,43± 0,06; 0,14 (0,49-0,38)	0,58± 0,00; 0,00 (0,58-0,58)	0,66± 0,08; 0,12 (0,74-0,59)

Continuación Tabla 5. 16, *P. (P.) subproducta* de Ullal de Baltasar, Amposta, Tarragona y 17, Laguna de Ontígola, Madrid; 18, *P. (P.) beckmanni* de Font del Rentador, Deiá, Mallorca; 19, *P. (P.) granjaensis* de la Granja de Esporles, Mallorca; 20, *P. (P.) artanensis* de la acequia de la ermita de Betlem, Artá, Mallorca; 21, *P. (P.) meloussensis* de la cala de la Macarella, Menorca; 22, *P. (P.) gasulli* del barranco de las Negras, Almería y 23, Rambla de los yesos, Alboloduy, Almería.

	16 Mean ± SD; CV (Max-Min) (n=3)	**17** Mean ± SD; CV (Max-Min) (n=3)	**18** Mean ± SD; CV (Max-Min) (n=3)	**19** Mean ± SD; CV (Max-Min) (n=1)	**20** Mean ± SD; CV (Max-Min) (n=3)	**21** Mean ± SD; CV (Max-Min) (n=4)	**22** Mean ± SD; CV (Max-Min) (n=4)	**23** Mean ± SD; CV (Max-Min) (n=6)
Po L	1,51± 0,80; 0,53 (2,32-1,00)	1,65± 0,19; 0,11 (1,81-1,48)	1,37± 0,04; 0,03 (1,41-1,34)	2,49	2,14± 0,23; 0,11 (2,36-1,97)	2,15± 0,08; 0,04 (2,23-2,05)	1,46± 0,28; 0,19 (1,69-1,11)	1,20± 0,23; 0,19 (1,54-0,98)
Po W	0,40± 0,19; 0,48 (0,59-0,26)	0,47± 0,15; 0,31 (0,56-0,32)	0,39± 0,04; 0,09 (0,43-0,37)	0,53	0,48± 0,04; 0,09 (0,52-0,45)	0,56± 0,07; 0,13 (0,64-0,58)	0,38± 0,11; 0,29 (0,49-0,26)	0,38± 0,04; 0,11 (0,43-0,34)
Ag, L	0,60± 0,31; 0,51 (0,88-0,34)	0,55± 0,20; 0,36 (0,75-0,44)	0,51± 0,03; 0,07 (0,54-0,48)	0,94	0,71± 0,09; 0,12 (0,79-0,64)	0,72± 0,21; 0,30 (0,93-0,46)	0,61± 0,13; 0,21 (0,73-0,47)	0,43± 0,09; 0,21 (0,54-0,32)
Cg, L	0,58± 0,20; 0,35 (0,79-0,47)	0,88± 0,06; 0,06 (0.93-0,83)	0,69± 0,04; 0,06 (0,72-0,65)	1,11	1,01± 0,05; 0,05 (1,06-0,99)	1,12± 0,21; 0,18 (1,35-0,89)	0,60± 0,08; 0,14 (0,67-0,51)	0,62± 0,15; 0,24 (0,81-0,50)
SR1 L	0,31± 0,13; 0,41 (0,41-0,19)	0,34± 0,14; 0,41 (0,42-0,20)	0,29± 0,07; 0,24 (0,36-0,24)	0,42	0,36± 0,14; 0,38 (0,50-0,28)	0,34± 0,05; 0,14 (0,40-0,30)	Ausente	Ausente
BC L	0,46± 0,19; 0,41 (0,65-0,34)	0,72± 0,06; 0,09 (0,78-0,68)	0,76± 0,16; 0,21 (0,88-0,60)	1,39	1,03± 0,12; 0,12 (1,14-0,92)	0,77± 0,12; 0,16 (0,91-0,63)	0,33± 0,09; 0,26 (0,41-0,22)	0,29± 0,06; 0,23 (0,41-0,24)
BC W	0,25± 0,00; 0,00 (0,25-0,25)	0,21± 0,08; 0,36 (0,28-0,15)	0,24± 0,10; 0,42 (0,33-0,15)	0,61	0,34± 0,02; 0,06 (0,35-0,32)	0,39± 0,06; 0,16 (0,43-0,30)	0,25± 0,05; 0,22 (0,30-0,18)	0,24± 0,05; 0,20 (0,30-0,18)
dBC L	0,55± 0,25; 0,45 (0,74-0,31)	0,55± 0,10; 0,18 (0,61-0,45)	0,69± 0,12; 0,17 (0,81-0,60)	0,60	0,89± 0,29; 0,33 (1,19-0,70)	0,56± 0,09; 0,15 (0,68-0,50)	0,43± 0,13; 0,31 (0,54-0,30)	0,29± 0,05; 0,18 (0,37-0,24)

Tabla 6. Medidas (en mm) del sistema genital masculino de las especies de *Pseudamnicola*: 1, *P. (C.) luisi* de fuente de La Gitana, La Peza, Granada; 2, *P. (C.) falkneri* de fuente de la Armada, Orce, Granada; 3, *P. (C.) manueli* del arroyo de La Garganta, Nava de San Pedro, Jaén; 4, *P. (C.) bareai* de la fuente de la Ermita de las Santas, Collados de la Sagra, Granada; 5, *P. (C.) marisolae* de la fuente de Pilar del Mono, Dúrcal, Granada; 6, *P. (C.) iruritai* de la fuente de Don Pedro, Loja, Granada; 7, *P. (C.) andalusica* de la fuente de La Salud, Albanchez de Mágina, Jaén.

	1 Mean ± SD; CV (Max-Min) (n=4)	**2** Mean ± SD; CV (Max-Min) (n=4)	**3** Mean ± SD; CV (Max-Min) (n=5)	**4** Mean ± SD; CV (Max-Min) (n=5)	**5** Mean ± SD; CV (Max-Min) (n=5)	**6** Mean ± SD; CV (Max-Min) (n=4)	**7** Mean ± SD; CV (Max-Min) (n=3)
Pr L	1,63± 0,07; 0,04 (1,70-1,56)	1,88± 0,06; 0,03 (1,96-1,84)	1,63± 0,62; 0,38 (2,50-0,98)	1,55± 0,21; 0,14 (1,78-1,33)	1,49± 0,32; 0,22 (1,85-1,17)	1,05± 0,11; 0,11 (1,10-0,89)	1,53± 0,27; 0,18 (1,80-1,20)
Pr W	0,63± 0,14; 0,22 (0,75-0,50)	0,49± 0,10; 0,20 (0,55-0,35)	0,47± 0,20; 0,43 (0,71-0,21)	0,49± 0,05; 0,10 (0,54-0,42)	0,43± 0,12; 0,28 (0,60-0,32)	0,33± 0,06; 0,18 (0,40-0,27)	0,57± 0,08; 0,13 (0,63-0,47)
P L	1,92± 0,08; 0,04 (2,00-1,83)	1,30± 0,14; 0,11 (1,45-1,17)	1,04± 0,12; 0,12 (1,13-0,81)	0,94± 0,19; 0,20 (1,20-0,77)	1,60± 0,19; 0,12 (1,87-1,40)	1,39± 0,11; 0,08 (1,49-1,30)	1,95± 0,31; 0,16 (2,27-1,60)
P W	0,35± 0,05; 0,13 (0,40-0,30)	0,27± 0,05; 0,18 (0,32-0,23)	0,24± 0,06; 0,24 (0,34-1,19)	0,21± 0,04; 0,21 (0,26-0,16)	0,35± 0,08; 0,23 (0,43-0,25)	0,29± 0,05; 0,18 (0,30-0,20)	0,49± 0,07; 0,15 (0,60-0,40)
PL/Head length	1,20± 0,25; 0,20 (1,43-0,92)	1,41± 0,21; 0,15 (1,66-1,24)	1,01± 0,23; 0,23 (1,25-0,70)	0,91± 0,33; 0,36 (1,39-0,57)	1,23± 0,18; 0,15 (1,40-1,02)	1,33± 0,35; 0,26 (1,65-1,00)	1,46± 0,22; 0,15 (1,75-1,26)

Continuación Tabla 6. 8, *P. (C.) astieri* de Source d'Argens, Brue-Aurillac, Var, France; 9, *P. (C.) hauffei* de fuente de los Nogales, Castellón; 10, *P. (C.) navasiana* de Fonnueva, Bulbuente, Zaragoza, 11, Pozo Azul en Covanera, Burgos y 12, Fuete María, Ontígola, Toledo; 13, *P. (C.) hinzi* de la balsa de Vargas, Borja, Zaragoza; 14, *P. (C.) hinzi* del parque fluvial de Calamocha, Teruel; 15, *P. (C.) ballestae* n. sp. de fuente en Jatar, Granada.

	8 Mean ± SD; CV (Max-Min) (n=4)	**9** Mean ± SD; CV (Max-Min) (n=4)	**10** Mean ± SD; CV (Max-Min) (n=4)	**11** Mean ± SD; CV (Max-Min) (n=3)	**12** Mean ± SD; CV (Max-Min) (n=3)	**13** Mean ± SD; CV (Max-Min) (n=5)	**14** Mean ± SD; CV (Max-Min) (n=3)	**15** Mean ± SD; CV (Max-Min) (n=4)
Pr L	1,67± 0,14; 0,08 (1,86-1,55)	1,37± 0,09; 0,06 (1,46-1,29)	1,36± 0,10; 0,07 (1,51-1,18)	1,38± 0,14; 0,10 (1,50-1,25)	1,71± 0,31; 0,18 (1,94-1,40)	1,38± 0,21; 0,15 (1,68-1,19)	1,46± 0,23; 0,16 (1,59-1,22)	2,19± 0,23; 0,10 (2,40-1,90)
Pr W	0,58± 0,09; 0,15 (0,69-0,52)	0,45± 0,05; 0,12 (0,51-0,41)	0,50± 0,05; 0,10 (0,57-0,43)	0,48± 0,10; 0,22 (0,56-0,38)	0,60± 0,09; 0,15 (0,69-0,54)	0,41± 0,03; 0,07 (0,45-0,38)	0,49± 0,14; 0,28 (0,63-0,40)	0,81± 0,13; 0,16 (0,97-0,70)
P L	1,26± 0,09; 0,07 (1,35-1,15)	1,28± 0,24; 0,19 (1,60-1,12)	1,39± 0,20; 0,14 (1,63-1,17)	1,56± 0,12; 0,07 (1,55-1,45)	1,66± 0,35; 0,21 (2,00-1,40)	0,86± 0,12; 0,14 (1,03-0,75)	1,19± 0,22; 0,19 (1,39-1,00)	1,58± 0,21; 0,13 (1,81-1,35)
P W	0,37± 0,03; 0,09 (0,40-0,33)	0,66± 0,12; 0,18 (0,75-0,50)	0,51± 0,07; 0,15 (0,61-0,40)	0,32± 0,04; 0,13 (0,36-0,29)	0,38± 0,11; 0,28 (0,49-0,32)	0,34± 0,03; 0,08 (0,36-0,30)	0,28± 0,08; 0,29 (0,35-0,21)	0,26± 0,03; 0,13 (0,29-0,23)
PL/Head length	1,02± 0,16; 0,16 (1,16-0,86)	0,91± 0,15; 0,17 (1,07-0,82)	0,96± 0,15; 0,15 (1,20-0,74)	1,62± 0,35; 0,22 (1,90-1,28)	1,30± 0,41; 0,32 (1,70-1,00)	0,72± 0,10; 0,15 (0,84-0,59)	0,94± 0,30; 0,32 (1,20-0,67)	1,07± 0,05; 0,04 (1,10-1,01)

Continuación Tabla 6. 16, *P. (P.) subproducta* de Ullal de Baltasar, Amposta, Tarragona y 17, Laguna de Ontígola, Madrid; 18, *P. (P.) beckmanni* de Font del Rentador, Deiá, Mallorca; 19, *P. (P.) granjaensis* de la Granja de Esporles, Mallorca; 20, *P. (P.) artanensis* de la acequia de la ermita de Betlem, Artá, Mallorca; 21, *P. (P.) meloussensis* de la cala de la Macarella, Menorca; 22, *P. (P.) gasulli* del barranco de las Negras, Almería y 23, Rambla de los yesos, Alboloduy, Almería.

	16 Mean ± SD; CV (Max-Min) (n=3)	**17** Mean ± SD; CV (Max-Min) (n=3)	**18** Mean ± SD; CV (Max-Min) (n=4)	**19** Mean ± SD; CV (Max-Min) (n=2)	**20** Mean ± SD; CV (Max-Min) (n=3)	**21** Mean ± SD; CV (Max-Min) (n=6)	**22** Mean ± SD; CV (Max-Min) (n=4)	**23** Mean ± SD; CV (Max-Min) (n=6)
Pr L	1,47± 0,22; 0,15 (1,70-1,35)	1,31± 0,16; 0,12 (1,46-1,18)	0,92± 0,13; 0,14 (1,06-0,77)	1,91± 0,29; 0,15 (2,07-1,74)	1,31± 0,18; 0,14 (1,46-1,14)	1,41± 0,13; 0,09 (1,58-1,20)	0,77± 0,10; 0,13 (0,89-0,67)	0,53± 0,13; 0,24 (0,70-0,34)
Pr W	0,52± 0,09; 0,18 (0,62-0,47)	0,50± 0,08; 0,16 (0,51-0,41)	0,37± 0,04; 0,11 (0,42-0,34)	0,66± 0,01; 0,01 (0,66-0,65)	0,47± 0,05; 0,10 (0,52-0,44)	0,45± 0,09; 0,21 (0,58-0,34)	0,33± 0,06; 0,18 (0,39-0,26)	0,26± 0,04; 0,17 (0,31-0,20)
P L	1,24± 0,39; 0,32 (1,59-0,90)	1,42± 0,15; 0,10 (1,50-1,27)	0,92± 0,03; 0,03 (0,95-0,89)	1,15± 0,24; 0,21 (1,28-1,01)	1,25± 0,23; 0,18 (1,48-1,12)	1,81± 0,50; 0,28 (2,29-1,15)	0,99± 0,20; 0,21 (1,20-0,76)	0,78± 0,19; 0,25 (1,12-0,58)
P W	0,49± 0,16; 0,33 (0,65-0,39)	0,44± 0,01; 0,03 (0,45-0,43)	0,37± 0,05; 0,14 (0,40-0,30)	0,35± 0,03; 0,08 (0,36-0,33)	0,51± 0,04; 0,08 (0,54-0,47)	0,53± 0,11; 0,21 (0,67-0,41)	0,14± 0,04; 0,27 (0,17-0,09)	0,14± 0,04; 0,28 (0,19-0,09)
PL/Head length	1,42± 0,10; 0,07 (1,52-1,36)	1,30± 0,45; 0,34 (1,69-0,90)	1,10± 0,24; 0,22 (1,31-0,80)	0,75± 0,42; 0,56 (0,98-0,51)	1,33± 0,26; 0,19 (1,54-1,09)	1,06± 0,24; 0,23 (1,32-0,84)	1,01± 0,16; 0,16 (1,17-0,82)	1,24± 0,40; 0,32 (1,60-0,53)

Tabla 7. Medidas (en mm) del sistema nervioso de las especies de *Pseudamnicola*: 1, *P. (C.) luisi* de fuente de La Gitana, La Peza, Granada; 2, *P. (C.) falkneri* de fuente de la Armada, Orce, Granada; 3, *P. (C.) manueli* del arroyo de La Garganta, Nava de San Pedro, Jaén; 4, *P. (C.) bareai* de la fuente de la Ermita de las Santas, Collados de la Sagra, Granada; 5, *P. (C.) marisolae* de la fuente de Pilar del Mono, Dúrcal, Granada; 6, *P. (C.) iruritai* de la fuente de Don Pedro, Loja, Granada; 7, *P. (C.) andalusica* de la fuente de La Salud, Albanchez de Mágina, Jaén.

	1 Mean ± SD; CV (Max-Min) (n=4)	**2** Mean ± SD; CV (Max-Min) (n=4)	**3** Mean ± SD; CV (Max-Min) (n=5)	**4** Mean ± SD; CV (Max-Min) (n=5)	**5** Mean ± SD; CV (Max-Min) (n=5)	**6** Mean ± SD; CV (Max-Min) (n=4)	**7** Mean ± SD; CV (Max-Min) (n=3)
LRCG	0,33± 0,03; 0,10 (0,38-0,28)	0,24± 0,07; 0,31 (0,34-0,19)	0,22± 0,03; 0,14 (0,26-0,17)	0,23± 0,02; 0,11 (0,25-0,18)	0,27± 0,04; 0,15 (0,32-0,20)	0,23± 0,02; 0,10 (0,26-0,21)	0,31± 0,01; 0,04 (0,32-0,30)
LLCG	0,31± 0,03; 0,10 (0,37-0,28)	0,21± 0,02; 0,09 (0,22-0,18)	0,23± 0,04; 0,17 (0,27-0,15)	0,21± 0,03; 0,13 (0,25-0,18)	0,26± 0,04; 0,15 (0,30-0,18)	0,22± 0,04; 0,18 (0,28-0,19)	0,29± 0,05; 0,18 (0,34-0,25)
LCC	0,16± 0,03; 0,18 (0,19-0,11)	0,11± 0,03; 0,26 (0,13-0,07)	0,15± 0,03; 0,16 (0,19-0,12)	0,13± 0,03; 0,22 (0,16-0,09)	0,11± 0,02; 0,19 (0,13-0,07)	0,14± 0,02; 0,17 (0,18-0,12)	0,11± 0,02; 0,15 (0,13-0,10)
LRPG	0,25± 0,08; 0,31 (0,40-0,18)	0,15± 0,03; 0,20 (0,19-0,13)	0,17± 0,03; 0,16 (0,23-0,15)	0,15± 0,04; 0,27 (0,21-0,11)	0,19± 0,03; 0,14 (0,23-0,15)	0,16± 0,03; 0,21 (0,19-0,12)	0,16± 0,01; 0,07 (0,17-0,15)
LLPG	0,21± 0,03; 0,15 (0,25-0,15)	0,17± 0,02; 0,13 (0,19-0,15)	0,21± 0,04; 0,21 (0,26-0,13)	0,20± 0,08; 0,39 (0,30-0,10)	0,19± 0,04; 0,23 (0,24-0,12)	0,15± 0,03; 0,20 (0,18-0,11)	0,13± 0,06; 0,42 (0,19-0,10)
LsupG	0,26± 0,01; 0,06 (0,28-0,24)	0,10± 0,02; 0,19 (0,12-0,08)	0,15± 0,03; 0,21 (0,19-0,10)	0,14± 0,03; 0,23 (0,21-0,11)	0,15± 0,05; 0,32 (0,23-0,09)	0,11± 0,03; 0,27 (0,14-0,07)	0,15± 0,00; 0,00 (0,15-0,15)
LsubG	0,14± 0,05; 0,38 (0,20-0,04)	0,11± 0,05; 0,42 (0,17-0,07)	0,10± 0,02; 0,19 (0,12-0,06)	0,11± 0,04; 0,33 (0,16-0,05)	0,16± 0,05; 0,29 (0,24-0,11)	0,09± 0,03; 0,29 (0,12-0,06)	0,10± 0,02; 0,17 (0,12-0,09)
LPsupC	0,25± 0,08; 0,32 (0,36-0,26)	0,23± 0,03; 0,13 (0,27-0,21)	0,26± 0,06; 0,24 (0,37-0,19)	0,22± 0,06; 0,28 (0,26-0,10)	0,28± 0,06; 0,21 (0,35-0,17)	0,21± 0,04; 0,21 (0,26-0,16)	0,24± 0,04; 0,17 (0,27-0,20)
LPsubC	0,07± 0,02; 0,27 (0,10-0,04)	0,03± 0,01; 0,20 (0,03-0,02)	0,07± 0,04; 0,56 (0,14-0,03)	0,05± 0,04; 0,92 (0,14-0,02)	0,07± 0,03; 0,39 (0,12-0,04)	0,08± 0,03; 0,41 (0,13-0,05)	0,06± 0,02; 0,38 (0,08-0,04)
RPG	0,41± 0,01; 0,02 (0,43-0,39)	0,48± 0,06; 0,13 (0,56-0,43)	0,44± 0,07; 0,16 (0,55-0,35)	0,42± 0,09; 0,21 (0,47-0,26)	0,45± 0,06; 0,13 (0,52-0,39)	0,44± 0,05; 0,11 (0,49-0,38)	0,43± 0,05; 0,12 (0,47-0,38)

Continuación Tabla 7. 8, *P. (C.) astieri* de Source d'Argens, Brue-Aurillac, Var, France; 9, *P. (C.) hauffei* de fuente de los Nogales, Castellón; 10, *P. (C.) navasiana* de Fonnueva, Bulbuente, Zaragoza, 11, Pozo Azul en Covanera, Burgos y 12, Fuete María, Ontígola, Toledo; 13, *P. (C.) hinzi* de la balsa de Vargas, Borja, Zaragoza; 14, *P. (C.) hinzi* del parque fluvial de Calamocha, Teruel; 15, *P. (C.) ballestae* n. sp. de fuente en Jatar, Granada.

	8 Mean ± SD; CV (Max-Min) (n=5)	**9** Mean ± SD; CV (Max-Min) (n=5)	**10** Mean ± SD; CV (Max-Min) (n=7)	**11** Mean ± SD; CV (Max-Min) (n=2)	**12** Mean ± SD; CV (Max-Min) (n=2)	**13** Mean ± SD; CV (Max-Min) (n=5)	**14** Mean ± SD; CV (Max-Min) (n=6)	**15** Mean ± SD; CV (Max-Min) (n=8)
LRCG	0,22± 0,02; 0,10 (0,24-0,20)	0,21± 0,02; 0,10 (0,34-0,19)	0,25± 0,03; 0,10 (0,29-0,23)	0,28± 0,01; 0,03 (0,28-0,27)	0,27± 0,03; 0,10 (0,28-0,25)	0,27± 0,04; 0,13 (0,31-0,24)	0,28± 0,03; 0,10 (0,31-0,24)	0,29± 0,02; 0,05 (0,31-0,27)
LLCG	0,22± 0,03; 0,15 (0,25-0,18)	0,22± 0,02; 0,10 (0,24-0,19)	0,23± 0,03; 0,11 (0,27-0,20)	0,26± 0,01; 0,03 (0,26-0,25)	0,28± 0,00; 0,00 (0,28-0,28)	0,25± 0,02; 0,07 (0,28-0,24)	0,27± 0,02; 0,08 (0,30-0,25)	0,28± 0,01; 0,03 (0,29-0,27)
LCC	0,11± 0,02; 0,19 (0,12-0,07)	0,15± 0,02; 0,10 (0,19-0,12)	0,12± 0,03; 0,22 (0,15-0,08)	0,14± 0,04; 0,33 (0,16-0,11)	0,12± 0,01; 0,08 (0,12-0,11)	0,11± 0,01; 0,12 (0,13-0,10)	0,16± 0,02; 0,12 (0,18-0,14)	0,17± 0,03; 0,18 (0,22-0,12)
LRPG	0,13± 0,03; 0,25 (0,18-0,10)	0,15± 0,02; 0,14 (0,16-0,12)	0,15± 0,03; 0,19 (0,21-0,13)	0,14± 0,04; 0,25 (0,16-0,12)	0,16± 0,04; 0,29 (0,18-0,13)	0,12± 0,02; 0,14 (0,15-0,11)	0,15± 0,04; 0,26 (0,19-0,09)	0,19± 0,03; 0,16 (0,22-0,14)
LLPG	0,17± 0,03; 0,19 (0,20-0,13)	0,23± 0,03; 0,14 (0,28-0,20)	0,15± 0,02; 0,16 (0,18-0,11)	0,15± 0,01; 0,06 (0,15-0,14)	0,14± 0,01; 0,07 (0,14-0,13)	0,18± 0,04; 0,24 (0,23-0,13)	0,13± 0,03; 0,23 (0,16-0,09)	0,20± 0,06; 0,28 (0,31-0,15)
LsupG	0,14± 0,03; 0,23 (0,17-0,11)	0,12± 0,02; 0,18 (0,15-0,10)	0,12± 0,04; 0,31 (0,16-0,06)	0,09± 0,04; 0,39 (0,11-0,07)	0,17± 0,02; 0,10 (0,18-0,16)	0,11± 0,03; 0,28 (0,15-0,07)	0,14± 0,03; 0,21 (0,19-0,12)	0,15± 0,03; 0,18 (0,19-0,11)
LsubG	0,11± 0,01; 0,10 (0,13-0,09)	0,12± 0,02; 0,18 (0,14-0,09)	0,13± 0,04; 0,32 (0,21-0,09)	0,14± 0,02; 0,13 (0,15-0,13)	0,14± 0,02; 0,13 (0,15-0,13)	0,12± 0,04; 0,35 (0,17-0,07)	0,13± 0,01; 0,10 (0,15-0,12)	0,13± 0,01; 0,10 (0,15-0,12)
LPsupC	0,18± 0,05; 0,30 (0,24-0,11)	0,29± 0,07; 0,26 (0,39-0,21)	0,20± 0,05; 0,25 (0,26-0,14)	0,18± 0,04; 0,20 (0,20-0,16)	0,35± 0,09; 0,25 (0,40-0,30)	0,33± 0,06; 0,18 (0,40-0,27)	0,27± 0,06; 0,22 (0,35-0,20)	0,30± 0,05; 0,16 (0,35-0,23)
LPsubC	0,07± 0,01; 0,15 (0,09-0,06)	0,10± 0,03; 0,32 (0,13-0,05)	0,05± 0,02; 0,32 (0,08-0,04)	0,05± 0,01; 0,20 (0,05-0,04)	0,07± 0,04; 0,51 (0,09-0,05)	0,05± 0,02; 0,40 (0,08-0,03)	0,05± 0,01; 0,23 (0,06-0,03)	0,10± 0,02; 0,20 (0,13-0,08)
RPG	0,40± 0,07; 0,13 (0,49-0,31)	0,52± 0,05; 0,10 (0,58-0,46)	0,42± 0,06; 0,14 (0,49-0,35)	0,44± 0,12; 0,28 (0,51-0,37)	0,52± 0,11; 0,21 (0,52-0,45)	0,58± 0,02; 0,03 (0,61-0,56)	0,48± 0,02; 0,04 (0,50-0,45)	0,47± 0,05; 0,11 (0,55-0,40)

Continuación Tabla 7. 16, *P. (P.) subproducta* de Ullal de Baltasar, Amposta, Tarragona y 17, Laguna de Ontígola, Madrid; 18, *P. (P.) beckmanni* de Font del Rentador, Deiá, Mallorca; 19, *P. (P.) granjaensis* de la Granja de Esporles, Mallorca; 20, *P. (P.) artanensis* de la acequia de la ermita de Betlem, Artá, Mallorca; 21, *P. (P.) meloussensis* de la cala de la Macarella, Menorca; 22, *P. (P.) gasulli* del barranco de las Negras, Almería y 23, Rambla de los yesos, Alboloduy, Almería.

	16 Mean ± SD; CV (Max-Min) (n=3)	**17** Mean ± SD; CV (Max-Min) (n=3)	**18** Mean ± SD; CV (Max-Min) (n=3)	**19** Mean ± SD; CV (Max-Min) (n=2)	**20** Mean ± SD; CV (Max-Min) (n=5)	**21** Mean ± SD; CV (Max-Min) (n=9)	**22** Mean ± SD; CV (Max-Min) (n=6)	**23** Mean ± SD; CV (Max-Min) (n=5)
LRCG	0,22± 0,05; 0,21 (0,24-0,17)	0,26± 0,02; 0,06 (0,27-0,24)	0,16± 0,04; 0,26 (0,19-0,12)	0,30± 0,04; 0,12 (0,28-0,27)	0,28± 0,03; 0,11 (0,33-0,26)	0,24± 0,03; 0,11 (0,29-0,21)	0,22± 0,01; 0,07 (0,24-0,20)	0,23± 0,03; 0,15 (0,26-0,18)
LLCG	0,21± 0,05; 0,23 (0,24-0,16)	0,24± 0,02; 0,06 (0,25-0,22)	0,15± 0,03; 0,02 (0,17-0,12)	0,33± 0,06; 0,19 (0,26-0,25)	0,26± 0,03; 0,11 (0,29-0,22)	0,23± 0,02; 0,09 (0,26-0,19)	0,21± 0,01; 0,07 (0,23-0,19)	0,23± 0,04; 0,16 (0,26-0,18)
LCC	0,14± 0,02; 0,17 (0,16-0,12)	0,10± 0,04; 0,46 (0,15-0,06)	0,08± 0,03; 0,34 (0,09-0,05)	0,09± 0,04; 0,39 (0,11-0,07)	0,10± 0,01; 0,14 (0,11-0,08)	0,10± 0,03; 0,31 (0,15-0,06)	0,06± 0,01; 0,24 (0,08-0,04)	0,14± 0,03; 0,25 (0,17-0,09)
LRPG	0,13± 0,01; 0,09 (0,14-0,12)	0,18± 0,02; 0,10 (0,20-0,16)	0,09± 0,03; 0,30 (0,12-0,07)	0,17± 0,02; 0,10 (0,18-0,16)	0,16± 0,04; 0,26 (0,19-0,10)	0,15± 0,04; 0,28 (0,19-0,09)	0,12± 0,03; 0,23 (0,15-0,09)	0,12± 0,02; 0,14 (0,15-0,11)
LLPG	0,12± 0,05; 0,44 (0,16-0,07)	0,16± 0,04; 0,23 (0,20-0,13)	0,05± 0,01; 0,24 (0,06-0,04)	0,20± 0,01; 0,05 (0,20-0,19)	0,17± 0,06; 0,39 (0,25-0,09)	0,20± 0,07; 0,36 (0,29-0,06)	0,11± 0,02; 0,21 (0,13-0,08)	0,11± 0,02; 0,21 (0,14-0,08)
LsupG	0,14± 0,05; 0,37 (0,18-0,09)	0,13± 0,03; 0,20 (0,16-0,10)	0,07± 0,02; 0,32 (0,09-0,05)	0,13± 0,01; 0,07 (0,13-0,12)	0,13± 0,02; 0,15 (0,15-0,11)	0,12± 0,02; 0,19 (0,16-0,10)	0,14± 0,03; 0,20 (0,16-0,09)	0,10± 0,02; 0,18 (0,13-0,09)
LsubG	0,11± 0,04; 0,37 (0,14-0,07)	0,14± 0,02; 0,16 (0,15-0,11)	0,07± 0,02; 0,24 (0,09-0,06)	0,13± 0,02; 0,14 (0,14-0,12)	0,11± 0,01; 0,10 (0,12-0,10)	0,11± 0,03; 0,28 (0,16-0,08)	0,08± 0,00; 0,05 (0,08-0,07)	0,10± 0,02; 0,22 (0,12-0,07)
LPsupC	0,23± 0,09; 0,39 (0,31-0,15)	0,26± 0,10; 0,40 (0,38-0,17)	0,18± 0,02; 0,13 (0,20-0,16)	0,45± 0,08; 0,18 (0,49-0,40)	0,28± 0,02; 0,08 (0,30-0,25)	0,41± 0,08; 0,20 (0,61-0,32)	0,26± 0,03; 0,13 (0,31-0,21)	0,26± 0,03; 0,11 (0,29-0,23)
LPsubC	0,03± 0,01; 0,38 (0,04-0,02)	0,04± 0,01; 0,28 (0,05-0,03)	0,02± 0,01; 0,28 (0,03-0,02)	0,07± 0,01; 0,14 (0,07-0,06)	0,05± 0,01; 0,19 (0,06-0,04)	0,05± 0,02; 0,40 (0,08-0,03)	0,04± 0,01; 0,31 (0,05-0,02)	0,02± 0,01; 0,24 (0,03-0,02)
RPG	0,45± 0,07; 0,15 (0,49-0,41)	0,44± 0,09; 0,20 (0,54-0,36)	0,53± 0,04; 0,08 (0,57-0,50)	0,60± 0,06; 0,11 (0,64-0,56)	0,50± 0,06; 0,12 (0,57-0,43)	0,60± 0,05; 0,08 (0,68-0,54)	0,50± 0,07; 0,14 (0,63-0,45)	0,54± 0,04; 0,08 (0,59-0,48)

Apéndice IV: Matriz de distancias genéticas

Tabla 1. Matriz de pares de distancias genéticas (distancias sin corregir) entre todas las especies analizadas, obtenidas para los genes COI (sector superior de cada celda) y 16S (número intermedio) y 28S (sector inferior de cada celda).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *P. (C.) andalusica*	2,8 1,5 0,1														
2. *P. (C.) astieri*	9,3 5,0 0,8	0,1 0,0 0,0													
3. *P. (C.) ballestae* n. sp.	7,0 2,2 0,3	10,2 5,6 0,6	0,0 0,0 0,0												
4. *P. (C.) bareai*	9,2 6,2 0,9	8,0 6,1 0,8	9,6 6,2 0,6	2,5 0,8 0,3											
5. *P. (C.) falkneri*	8,7 3,1 0,7	8,9 4,1 0,8	9,2 3,8 0,4	8,4 6,3 0,8	1,4 0,6 0,1										
6. *P. (C.) hauffei*	10,5 5,0 0,6	7,4 2,3 0,3	12,0 5,7 0,3	8,1 5,0 0,5	8,9 3,5 0,5	0,2 0,2 0,0									
7. *P. (C.) hinzi*	9,0 4,5 0,5	9,6 4,2 0,6	10,1 5,1 0,2	8,8 5,7 0,6	8,4 4,6 0,4	9,1 4,0 0,3	0,5 0,3 0,0								
8. *P. (C.) iruritai*	5,5 1,7 0,4	8,0 4,0 0,8	6,5 2,2 0,3	8,0 6,2 0,9	7,8 2,6 0,7	9,4 4,0 0,6	8,8 4,5 0,5	0,1 0,0 0,0							
9. *P. (C.) luisi*	7,0 3,0 0,2	10,6 4,8 0,7	7,1 3,3 0,1	10,1 6,4 0,7	10,0 3,7 0,5	11,9 4,9 0,4	10,7 4,5 0,3	6,6 2,1 0,2	0,6 0,5 0,0						
10. *P. (C.) manueli*	10,1 5,6 0,8	9,5 5,4 0,8	10,6 5,6 0,6	6,7 2,4 0,3	8,7 5,5 0,7	9,1 4,3 0,5	9,3 5,2 0,6	9,4 5,6 0,8	11,4 6,0 0,7	2,1 0,6 0,1					
11. *P. (C.) marisolae*	6,7 3,2 0,2	10,3 5,1 0,7	7,6 3,9 0,1	9,8 6,8 0,8	9,5 3,5 0,6	10,7 5,0 0,5	10,2 4,6 0,3	6,5 2,9 0,2	6,2 2,3 0,0	10,3 6,1 0,7	0,6 0,2 0,1				
12. *P. (C.) navasiana*	10,3 5,2 0,6	8,6 2,3 0,3	12,0 5,9 0,4	8,9 5,3 0,6	9,3 3,5 0,5	6,2 1,0 0,1	8,8 4,4 0,4	9,9 4,3 0,7	11,6 5,2 0,5	9,1 4,4 0,5	11,3 5,3 0,5	1,2 0,3 0,1			
13. *P.? (P.) gasulli*	10,6 8,7 5,5	10,4 7,1 6,0	10,4 9,4 5,5	11,1 8,6 6,0	11,2 7,3 5,6	12,3 6,6 5,7	10,5 7,7 5,6	10,3 8,2 5,7	11,7 8,2 5,5	11,8 8,5 5,7	11,2 8,3 5,5	11,6 6,8 5,7	1,2 0,8 1,1		
14. *Peringia ulvae*	18,4 10,3 10,2	17,0 10,2 10,1	16,9 10,6 10,3	18,9 10,9 10,4	17,9 10,4 10,6	19,0 9,8 10,2	18,0 10,1 10,4	16,3 10,2 10,4	18,4 10,3 10,3	19,6 10,7 10,4	18,6 11,1 10,3	19,3 9,7 10,1	17,3 11,0 13,6	0,0 0,0 0,0	
15. *Mercuria emiliana*	16,4 10,9 11,7	15,4 9,6 11,7	15,6 11,8 11,7	15,0 11,9 11,9	16,4 10,2 11,9	16,8 9,1 11,7	16,5 9,3 11,7	14,9 10,4 11,9	16,8 10,0 11,8	15,5 11,2 11,9	16,6 10,7 11,8	16,0 9,3 11,6	14,1 10,9 14,2	17,8 9,2 6,5	0,0 0,0 0,0

Continuación Tabla 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
16. *P. (P.) artanensis*	14,7 8,8 2,8	13,7 7,0 3,0	15,0 9,1 2,8	14,3 8,9 2,9	14,1 7,5 3,0	13,9 7,2 2,8	14,1 7,4 2,7	14,1 8,6 3,1	14,7 7,8 2,9	15,1 8,7 2,7	15,8 9,2 3,0	14,1 7,0 2,8	11,6 6,4 6,9	17,9 9,6 11,7	17,0 10,2 12,5
17. *P. (P.) beckmanni*	14,8 8,7 2,1	13,8 6,9 2,1	15,0 9,0 1,9	14,3 8,8 2,2	14,1 7,5 2,1	14,2 7,1 1,8	14,4 7,3 1,9	14,4 8,6 2,2	15,0 7,9 2,0	15,1 8,6 2,0	16,3 9,2 2,0	14,4 6,9 1,9	11,8 6,3 6,0	17,8 9,5 11,4	17,4 10,2 12,8
18. *P. (P.)* sp. 2	13,2 8,9 2,8	12,3 7,5 2,9	13,2 9,3 2,6	12,7 8,9 2,9	12,4 7,6 2,9	13,2 7,3 2,7	13,1 7,4 2,5	12,4 8,8 2,9	13,9 8,3 2,7	13,3 8,6 2,8	14,8 9,0 2,8	13,3 7,2 2,7	10,7 6,1 6,0	16,5 9,7 11,4	16,1 10,2 12,8
19. *P. (P.)* sp. 4	14,2 9,1 3,1	14,3 7,6 3,5	14,7 9,4 3,1	13,9 9,1 3,4	14,1 7,8 3,5	13,9 7,4 3,3	13,7 7,6 3,1	14,4 8,9 3,4	15,2 8,4 3,2	14,7 8,9 3,3	15,3 9,5 3,2	15,1 7,3 3,4	12,1 6,2 6,1	17,9 9,7 11,7	16,8 10,6 13,2
20. *P. (P.) granjaensis*	15,0 8,6 2,1	14,1 6,8 2,1	15,1 9,0 1,9	14,5 8,7 2,1	14,2 7,4 2,0	14,4 7,0 1,8	14,5 7,2 1,9	14,6 8,5 2,2	15,2 7,9 2,0	15,2 8,6 1,9	16,6 9,1 2,0	14,5 6,9 1,8	12,0 6,2 6,0	18,1 9,4 11,2	17,4 10,2 12,6
21. *P. (P.)* sp. 1	14,8 8,5 2,3	14,9 6,8 2,3	14,6 8,9 2,1	14,4 9,1 2,3	13,8 7,1 2,2	14,7 7,2 2,0	14,2 8,2 2,1	14,4 8,1 2,4	15,1 8,0 2,2	14,9 8,6 2,1	15,6 9,0 2,2	14,5 7,1 2,0	11,4 7,1 5,9	17,8 9,8 11,1	16,9 10,8 12,6
22. *P. (P.) macrostoma*	13,9 9,0 2,5	13,6 6,4 2,5	14,5 9,3 2,3	13,8 8,7 2,5	12,8 7,5 2,5	14,1 6,8 2,2	14,2 7,8 2,1	13,4 8,5 2,6	13,8 8,6 2,4	14,0 8,5 2,3	13,8 9,8 2,5	13,4 6,7 2,3	11,9 6,5 5,9	18,2 10,0 11,4	16,3 11,0 12,8
23. *P. (P.) meloussensis*	14,3 8,3 2,4	14,8 6,2 2,6	14,9 8,8 2,4	14,2 8,8 2,7	13,9 6,8 2,6	14,2 6,6 2,4	14,4 7,4 2,4	13,1 7,5 2,7	14,7 7,6 2,5	14,7 8,7 2,5	15,2 8,9 2,6	14,5 6,5 2,4	12,4 6,4 6,5	18,8 9,6 11,5	16,2 10,4 12,9
24. *P. (P.)* sp. 3	14,3 8,8 3,2	14,2 7,3 3,6	15,1 9,2 3,3	13,9 8,9 3,5	13,9 7,6 3,5	14,3 7,1 3,3	14,1 7,5 3,2	14,0 8,7 3,5	15,0 8,0 3,3	14,9 8,8 3,3	15,4 9,0 3,3	14,2 7,1 3,4	12,3 6,3 6,3	18,2 9,7 11,5	17,1 10,4 13,1
25. *P. (P.) orsinii*	14,2 9,5 2,6	13,4 7,4 2,6	14,6 9,9 2,4	13,9 9,1 2,6	13,7 8,2 2,5	13,9 7,5 2,3	13,9 7,4 2,2	13,7 9,1 2,7	14,7 8,1 2,5	14,7 9,1 2,4	15,1 9,1 2,5	13,6 7,5 2,3	11,9 6,5 5,6	17,2 10,2 11,5	17,2 9,8 12,5
26. *P. (P.) subproducta*	14,1 8,9 2,4	14,7 6,7 2,6	15,3 9,1 2,4	14,2 9,3 2,6	14,3 7,6 2,5	14,7 7,4 2,3	14,4 8,3 2,4	14,3 8,1 2,7	15,3 8,2 2,5	14,7 9,4 2,4	15,0 9,0 2,5	14,7 7,3 2,3	12,4 7,0 6,4	17,6 10,3 11,5	17,5 11,2 12,8
27. *P. (P.) moussoni*	14,2 9,3 2,4	14,3 7,6 2,4	13,9 9,7 2,2	13,3 10,0 2,3	12,5 8,3 2,3	13,4 8,2 2,1	14,1 8,6 2,0	12,9 8,7 2,5	14,3 8,4 2,3	14,2 9,7 2,1	14,1 9,7 2,3	14,4 8,0 2,1	11,1 7,2 6,0	19,0 9,6 11,6	17,0 11,0 12,8

Continuación Tabla 1.

	16	17	18	19	20	21	22	23	24	25	26	27
16. *P. (P.) artanensis*	0,0 0,0 0,0											
17. *P. (P.) beckmanni*	1,6 0,5 1,5	0,6 0,4 0,1										
18. *P. (P.)* sp. 2	5,7 1,8 2,2	5,4 1,7 1,4	1,6 1,0 1,1									
19. *P. (P.)* sp. 4	7,5 1,5 2,4	7,9 1,5 1,9	6,9 1,2 1,8	0,4 0,1 0,5								
20. *P. (P.) granjaensis*	1,7 0,5 1,4	0,5 0,3 0,1	5,6 1,5 1,4	8,0 1,3 2,0	0,2 0,1 0,0							
21. *P. (P.)* sp. 1	7,0 2,0 1,6	7,1 2,1 0,4	8,0 2,5 1,5	8,9 2,0 2,0	7,1 2,0 0,4	0,0 0,0 0,0						
22. *P. (P.) macrostoma*	7,4 2,2 1,7	7,8 2,0 0,7	7,8 2,3 1,7	9,7 2,2 1,8	7,9 1,8 0,7	6,7 2,4 0,9	0,0 0,0 0,1					
23. *P. (P.) meloussensis*	6,3 2,0 1,4	6,5 2,0 0,9	7,7 2,5 2,0	9,4 2,1 2,3	6,7 1,8 0,9	6,4 1,5 1,2	6,8 1,8 1,1	1,3 0,5 0,4				
24. *P. (P.)* sp. 3	6,6 1,9 2,4	6,3 1,8 1,8	6,4 1,3 1,8	8,5 1,2 1,8	6,4 1,5 1,8	6,8 2,3 2,1	7,7 2,1 1,9	7,1 2,3 2,3	5,3 1,0 0,6			
25. *P. (P.) orsinii*	6,5 2,2 1,9	6,0 2,5 0,9	3,9 1,9 1,4	7,0 1,7 1,8	6,1 2,2 0,9	7,8 3,0 1,0	7,1 3,2 1,0	7,8 3,4 1,6	6,1 1,9 1,6	0,0 0,0 0,1		
26. *P. (P.) subproducta*	7,3 3,1 1,8	7,7 3,0 0,9	8,3 2,8 2,1	10,0 2,4 2,3	7,9 2,8 0,8	7,3 1,7 1,0	7,6 2,5 1,0	5,6 1,5 1,1	8,5 2,7 2,2	8,7 3,4 1,8	1,4 0,6 0,2	
27. *P. (P.) moussoni*	7,6 2,8 1,7	7,9 2,6 0,8	8,1 3,0 1,5	8,5 3,2 1,8	8,1 2,4 0,8	6,1 3,4 1,0	6,5 3,0 0,6	6,2 2,7 1,1	7,8 3,4 1,6	8,4 3,6 0,6	6,5 3,7 1,2	0,0 0,0 0,0